SECTION 2.6.4—PHARMACOKINETICS WRITTEN SUMMARY

BICTEGRAVIR/EMTRICITABINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (B/F/TAF FDC)

Gilead Sciences

[redacted] **20**

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
AhR	aryl hydrocarbon receptor
ARV	antiretroviral
AUC	area under the plasma concentration versus time curve
AUC_{0-t}	area under the time-concentration curve from time zero to last measured time-point
AUC_{inf}	area under the concentration versus time curve extrapolated to infinite time, calculated as $AUC_{last} + (C_{last}/\lambda_z)$
AUCR	AUC ratio
B/F/TAF	bictegravir/emtricitabine/tenofovir alafenamide (coformulated)
B/P	blood to plasma ratio
BCRP	breast cancer resistance protein
BDC	bile duct cannulated
BIC	bictegravir (GS-9883)
BLQ	below the limit of quantitation
BNPP	bis-p-nitrophenyl phosphate
BSEP	bile salt export pump
CAR	constitutive androstane receptor
CatA	cathepsin A
CCM	cell culture medium
cDNA	complementary DNA
CES1	carboxylesterase 1
CHB	chronic hepatitis B
CHO	Chinese hamster ovary
CL	clearance
CL/F	apparent oral clearance after administration of the drug: $CL/F = Dose/AUC_{inf}$, where "Dose" is the dose of the drug
C_{last}	last observed quantifiable concentration of the drug in plasma
C_{max}	maximum observed concentration of drug in plasma
CNS	central nervous system
COBI	cobicistat
CsA	cyclosporine (cyclosporin A)
CSF	cerebrospinal fluid
CYP	cytochrome P450 enzyme
DDI	drug-drug interaction
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated; Genvoya®)
EC_{50}	half-maximal effective concentration
EMA	European Medicines Agency
EVG	elvitegravir (Vitekta®)
F/TAF	emtricitabine/tenofovir alafenamide

FDA	Food and Drug Administration
FDC	fixed-dose combination
FMO	flavin monooxygenase
FTC	emtricitabine
FTC/RPV/TAF	emtricitabine/rilpivirine/tenofovir alafenamide (coformulated; Odefsey®)
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada®)
GD	gestation day
GI	gastrointestinal
GLP	Good Laboratory Practice
GFR	glomerular filtration rate
HBV	hepatitis B virus
HIV-1	human immunodeficiency virus type 1
HPLC	high-performance liquid chromatography
HPMC	hydroxypropyl methyl cellulose
HRMS	high resolution mass spectrometry
IC_{50}	half-maximal inhibitory concentration
ICH	International Council for Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
IV	intravenous
K_i	kinetic inhibition constant
λ_z	terminal elimination rate constant, estimated by linear regression of the terminal elimination phase of the log plasma/serum concentration of drug versus time curve of the drug
LC	liquid chromatography
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LC-UV	liquid chromatography coupled to ultraviolet detection
LLOQ	lower limit of quantitation
LSC	liquid scintillation counting
MATE	multidrug and toxin extrusion
MATEx	multidrug and toxin extrusion x
MDCK	Madin-Darby canine kidney
MDCKII	Madin-Darby canine kidney strain II
mRNA	messenger RNA
MRP	multidrug resistance-associated protein
MRP2	multidrug resistance-associated protein 2
MRP4	multidrug resistance-associated protein 4
MRT	mean residence time
MS	mass spectrometry
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
ND	not detectable
NR	not represented (tissue not present in section)
NRTI	nucleoside reverse transcriptase inhibitor

NS3	nonstructural protein 3
NtRTI	nucleotide reverse transcriptase inhibitor
NZW	New Zealand White
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
OGTT	oral glucose tolerance test
P_{app}	apparent permeability coefficient
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PD	pharmacodynamics(s)
PEG	polyethylene glycol
P-gp	P-glycoprotein
PI	protease inhibitor
PK	pharmacokinetic(s)
PXR	pregnane X receptor
QWBA	quantitative whole body autoradiography
RBC	red blood cell
RFD	radio flow-through detector
RNA	ribonucleic acid
RPV	rilpivirine
RT	reverse transcriptase
RT-PCR	reverse transcriptase polymerase chain reaction
SD	standard deviation
$t_{1/2}$	estimate of the terminal elimination half-life of the drug in plasma/serum, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
TAF	tenofovir alafenamide
TDF	tenofovir disoproxil fumarate
TFV	tenofovir
TFV-DP	tenofovir diphosphate
TFV-MP	tenofovir monophosphate
TK	toxicokinetic(s)
T_{last}	time (observed time point) of C_{last}
T_{max}	time (observed time point) of C_{max}
UDP	uridine diphosphate
UDPGA	uridine diphosphate glucuronic acid
UGT	uridine diphosphate glucuronosyltransferase
UGT1A1	uridine diphosphate glucuronosyltransferase 1A1
ULOQ	upper limit of quantitation
vs	versus
V_{ss}	volume of distribution at steady state

PHARMACOKINETIC ABBREVIATIONS

λ_z	terminal elimination rate constant, estimated by linear regression of the terminal elimination phase of the log plasma/serum concentration of drug versus time curve of the drug
AUC	area under the plasma concentration versus time curve
$AUC_{0-\infty}$, AUC_{inf}	area under the plasma concentration versus time curve extrapolated to infinite time, calculated as $AUC_{0-last} + (C_{last}/\lambda_z)$
AUC_{x-xx}	partial area under the plasma/serum concentration versus time curve from time “x” to time “xx”
CL	clearance
CL/F	apparent oral clearance after administration of the drug: $CL/F = Dose/AUC_{inf}$, where “Dose” is the dose of the drug
C_{last}	last observed quantifiable concentration of the drug in plasma
C_{max}	maximum observed concentration of drug in plasma
C_x	plasma concentration at time "x" (default units are hours)
F	estimated oral bioavailability of the drug (%)
$t_{1/2}$	estimate of the terminal elimination half-life of the drug in plasma/serum, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
T_{last}	time (observed time point) of C_{last}
T_{max}	time (observed time point) of C_{max}
V_{ss}	volume of distribution at steady state

NOTE TO REVIEWER

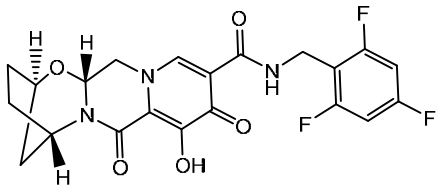
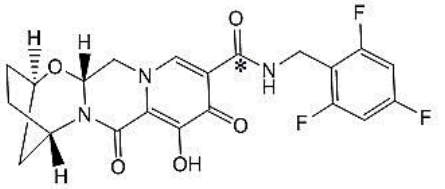
BIC

The structure of BIC and related compounds are illustrated in Table 1. The following conversions are provided to aid the reviewer.

1 µM BIC (GS-9883) = 0.449 µg/mL

1 ng/mL BIC = 2.23 nM

Table 1. Names and Structures of BIC and Related Compounds

Name	Alternative Names	Identity	Structure
BIC	Bictegravir, GS-9883	Parent Compound	
[^{14}C]BIC	[10-^{14}C]BIC	Radiolabeled parent	 (* = ^{14}C)

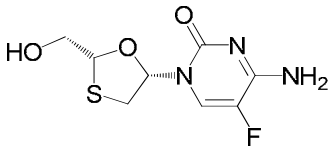
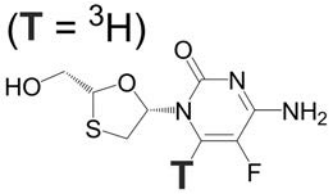
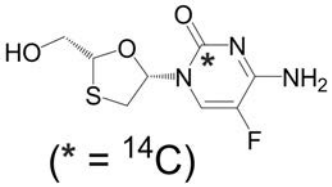
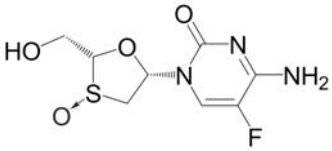
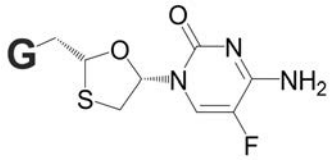
FTC

Structures of emtricitabine (FTC) and related compounds are illustrated in Table 2. The following conversions are provided to aid the reviewer:

1 µM FTC (GS-9019) = 0.247 µg/mL

1 ng/mL FTC = 4.05 nM

Table 2. Names and Structures of FTC and Related Compounds

Name	Alternative Names	Identity	Structure
FTC	Emtricitabine, GS-9019, TP-0006, 524W91, Emtriva, Coviracil	Parent Compound	
[^{3}H]FTC	[6-^{3}H]FTC	Radiolabeled parent	
[^{14}C]FTC	[2-^{14}C]FTC	Radiolabeled parent	
M1, M2	M950, 3742W92, 3743W92	3'-Sulfoxide metabolites (2 diastereomers)	
M3		2'-O-Glucuronide metabolite	

G = Glucuronic acid

TAF

Structures of tenofovir (TFV), tenofovir alafenamide (TAF), and related compounds are illustrated in Table 3. The following conversions are provided to aid the reviewer:

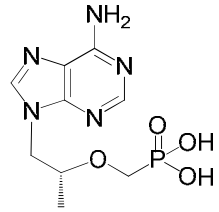
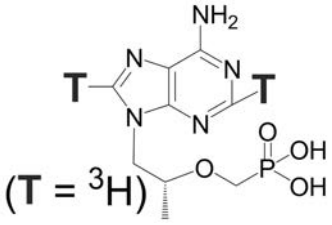
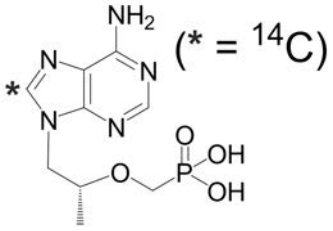
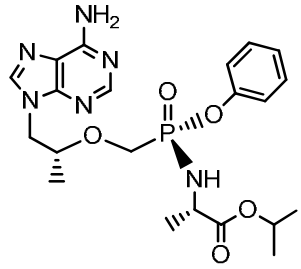
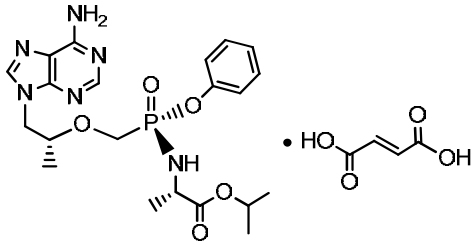
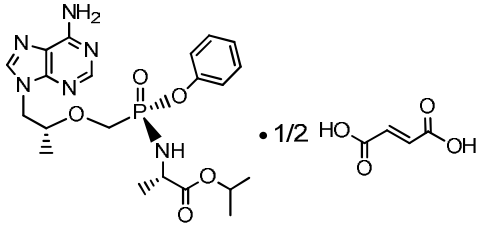
1 µM TFV (GS-1278) = 0.287 µg/mL

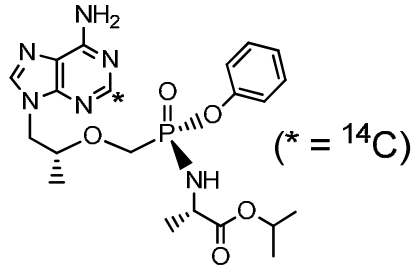
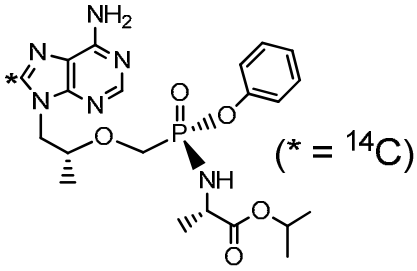
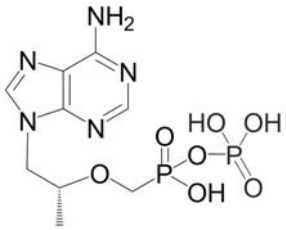
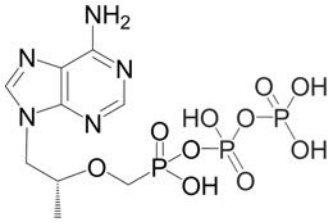
1 ng/mL TFV = 3.48 nM

1 µM TAF (GS-7340) = 0.477 µg/mL

1 ng/mL TAF = 2.10 nM

Table 3. Names and Structures of TFV, TAF, and Related Compounds

Name	Alternative Names	Identity	Structure
TFV	Tenofovir, GS-1278, R-PMPA	Parent Compound	
[^{3}H]TFV	[Adenine-2,8-^{3}H]TFV	Radiolabeled Parent	
[^{14}C]TFV	[Adenine-8-^{14}C]TFV	Radiolabeled Parent	
TAF (free base)		Parent Compound, free base	
GS-7340-02		Parent Compound, monofumarate	
GS-7340-03		Parent Compound, hemifumarate	

Name	Alternative Names	Identity	Structure
[^{14}C]TAF	[Adenine-2-^{14}C]TAF	Radiolabeled Parent	
	[Adenine-8-^{14}C]TAF	Radiolabeled Parent	
GS-342031	GS-77389, PMPAp, TFV-MP	Monophosphorylated Anabolite of Parent	
GS-077635	PMPApp, TFV-DP	Diphosphorylated Anabolite of Parent	

1. BRIEF SUMMARY

This dossier is being submitted in support of a marketing application for a fixed dose combination (FDC) of bictegravir (BIC, B, GS-9883), emtricitabine (FTC, F, GS-9019) and tenofovir alafenamide (TAF, GS-7340): the B/F/TAF (50/200/25 mg) FDC. Bictegravir is a low molecular weight HIV-1 integrase strand transfer inhibitor (INSTI) active against a broad panel of HIV-1 viral lab strains and clinical isolates and is fully active against a panel of mutant viruses with resistance to nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitors (NNRTI) and protease inhibitors (PI). Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) and is approved for the treatment of HIV-1 infection as a single agent (Emtriva®) for use in combination with other ARVs for the treatment of HIV-1 infection, and in the FDC products Truvada® (FTC/tenofovir disoproxil fumarate [TDF]), Atripla® (efavirenz/FTC/TDF), Complera®/ Eviplera® (FTC/rilpivirine [RPV]/TDF), Stribild® (elvitegravir [EVG; E]/cobicistat [COBI; C]/ FTC/TDF), Genvoya® (E/C/F/TAF), Descovy® (F/TAF) and Odefsey® (FTC/RPV/TAF). Tenofovir alafenamide is a prodrug of tenofovir (TFV), a nucleotide reverse transcriptase inhibitor (NtRTI). Tenofovir alafenamide is approved for the treatment of HIV-1 infection in the FDC products Genvoya®, Descovy® and Odefsey®. Tenofovir alafenamide is also approved for the treatment of hepatitis B virus (HBV) infection as a single agent (Vemlidy®). Information from all nonclinical studies with FTC, and TAF/TFV should be considered in the context of their clinical experience within ARV combination therapy for the treatment of HIV-1 infection.

BIC

Bictegravir is a potent HIV-1 integrase strand transfer inhibitor. BIC was highly permeable and showed efflux transport in vitro. Nonclinical studies to characterize the absorption and disposition of BIC have been performed in rats and monkeys. Bictegravir systemic plasma clearance (CL) was low in nonclinical species (0.1% to 1.3% of hepatic blood flow). Bictegravir volume of distribution (V_{ss}; 0.09 to 0.22 L/kg) in animals was lower than total body water. Bictegravir showed moderate to high oral bioavailability (42% to 74%) in nonclinical species. Overall, these data support high intestinal absorption for BIC in humans. Bictegravir plasma exposure increased following repeat oral administration of BIC; the increases were less than dose proportional. In rats, females had higher BIC exposures than males (2-to 3-fold at the high 300 mg/kg/day dose). None to slight accumulation (up to 3-fold) of BIC was observed in rats after repeat dosing. In cynomolgus monkeys, gender-based differences were less than 2-fold in BIC exposures and no accumulation (< 2-fold) of BIC was observed after repeat dosing.

Bictegravir was highly bound to plasma proteins in all species tested (> 98% bound) and was 99.75% bound in humans. Bictegravir has minimal binding to erythrocytes; the blood to plasma BIC concentration ratio was close to 0.6 in all species.

[^{14}C]BIC was widely distributed in tissues following oral administration in non-pigmented and pigmented rats. [^{14}C]BIC-derived radioactivity in most tissues reached maximum concentration by 1 hour post dose. Steady excretion of BIC radioactive equivalents in urine and feces coupled with declines in all tissues was consistent with the long mean residence time (MRT) of BIC in

rats (46 hours) and suggested no irreversible binding. [^{14}C]BIC-derived radioactivity poorly crossed the blood to brain barriers (< 4% relative to blood). [^{14}C]BIC-derived radioactivity was not selectively bound to melanin containing tissues.

Bictegravir was mainly eliminated by hepatic metabolism followed by excretion into feces and urine. Metabolic pathways included hydroxylation, oxidative defluorination, direct glucuronidation, and oxidation followed by phase II conjugation. There were no unique human metabolites; all human metabolites were also found in nonclinical species.

Bictegravir metabolism was predominantly mediated by cytochrome P450 (CYP)3A and uridine diphosphate glucuronosyltransferase (UGT)1A1. Bictegravir had little or no inhibitory effect (50% inhibitory concentration [IC_{50}] > 100 μM) on the activities of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A or UGT1A1. Bictegravir showed no time-dependent inhibition against CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6. Bictegravir was a very weak mechanism based inhibitor of CYP3A (K_I > 100 μM). Bictegravir is unlikely to be a clinically relevant mechanism-based inhibitor of CYP3A because the computed K_I greatly exceeds the unbound C_{max} in human plasma (~34 nM). Bictegravir was not an inducer of CYPs 1A2, 2C8 or 2C9. BIC was a weak inducer of CYP3A4 as concentration-dependent CYP3A4 messenger RNA (mRNA) increases were observed up to 16.7-fold at 60 μM BIC. Bictegravir presents a low potential as an inducer at clinically relevant concentrations because it is highly bound to plasma proteins (human > 99%). The low potential for clinically meaningful DDIs was confirmed in dedicated clinical studies; the plasma PK of CYP3A4 sensitive substrate midazolam and partial substrates velpatasvir, voxilaprevir, norgestimate, and ethinyl estradiol PK were each unaffected following repeat dose administration of B/F/TAF FDC. Further, repeat dose administration of BIC resulted in no change in BIC elimination half-life, suggesting a lack of autoinduction.

Bictegravir was a substrate for intestinal efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) and its intestinal absorption may be decreased by inducers or increased by coadministered inhibitors of P-gp and BCRP. Bictegravir was not an inhibitor of the hepatic transporters organic anion transporting polypeptide (OATP)1B1 or OATP1B3, organic cation transporter (OCT)1, and bile salt export pump (BSEP), or the renal transporters OAT1 and OAT3. BIC was an inhibitor of renal efflux transporter multidrug and toxin extrusion (MATE)1 with an 50% inhibitory concentration (IC_{50}) value of 8.0 μM. Bictegravir was an inhibitor of renal uptake transporter, OCT2, with an IC_{50} value of 0.42 μM. Clinical studies with B/F/TAF FDC and metformin coadministration showed a minimal change in the plasma exposure (AUC) of metformin (39% increase) with no effect on the pharmacodynamics (PD) end points such as glucose metabolism, and active GLP-1 and lactate levels after oral glucose tolerance test (OGTT).

FTC

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI). Nonclinical studies to characterize the absorption and disposition of FTC have been performed in mice, rats, and primates. The PK studies are listed in the overview table (m2.6.5, Section 1), and study details are provided in the individual study overview tables in m2.6.5. The tabulated summaries in m2.6.5 also provide toxicokinetic (TK) data for studies described in m2.6.6.

In mice, rats, and cynomolgus monkeys, FTC was rapidly and extensively absorbed with oral bioavailability ranging from 58% to 97%. In general, there were no differences in PK profiles following single and multiple dosing. Systemic exposure to FTC (C_{max} and AUC) increased approximately proportionally with dose and was similar between males and females. With chronic dosing, somewhat higher exposures were observed in the mouse and rat studies when compared to short term dosing; however, there was no evidence of accumulation in the monkey studies.

Emtricitabine is widely distributed throughout the body, with a volume of distribution similar to that of total body water. After oral administration, the highest concentrations of FTC were found in the kidneys, intestine, and liver, and exceeded those in plasma, while concentrations in central nervous system (CNS) tissues were less than 10% of those in plasma. Emtricitabine was also readily transferred across the placenta. Emtricitabine is almost completely eliminated within 72 hours following dosing, with no evidence of tissue accumulation. Emtricitabine does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. The total body clearance of FTC exceeds the glomerular filtration rate, suggesting that the drug is actively secreted by the kidney.

Metabolism is a minor route of elimination and is similar in humans and monkeys. It includes oxidation of the thiol moiety (Phase 1 metabolism) to form the 3'-sulfoxide diastereomers (M1 and M2) and conjugation with glucuronic acid (Phase 2 metabolism) to form the 2'-O-glucuronide (M3). The most abundant metabolite was one of the 3'-sulfoxides (M1 or M2). Several minor metabolites account for < 2% of the dose and are eliminated primarily in the urine. Importantly, FTC is not converted to 5-fluorouracil. Oxidation of FTC is largely catalyzed by CYP3A, but flavin monooxygenase (FMO) enzymes may also play a role. Emtricitabine does not inhibit human CYP and demonstrates no liability to be an inducer.

TAF

Tenofovir alafenamide is a prodrug of TFV, a nucleotide reverse transcriptase inhibitor (NtRTI). In target cells, TAF is rapidly hydrolyzed to TFV and sequentially phosphorylated to the pharmacologically active metabolite tenofovir diphosphate (TFV-DP). Tenofovir diphosphate is an inhibitor of HIV-1 reverse transcriptase (RT) and HBV RT that terminates the elongation of the viral DNA chain {Cherrington 1995, Yokota 1994}. Because TAF is more stable in plasma than tenofovir disoproxil fumarate (TDF), higher intracellular levels of TFV-DP are formed and approximately 90% lower circulating levels of TFV relative to TDF are observed when TAF is administered at approximately one tenth the TDF dose {Birkus 2007a, Birkus 2008, Lee 2005, Markowitz 2011}. This distinct metabolism of TAF offers the potential for an improved clinical profile compared with TDF.

Tenofovir alafenamide has minimal interaction with typical xenobiotic metabolizing enzymes and is primarily hydrolyzed by carboxylesterase 1 (CES1) in primary human hepatocytes {Birkus 2007a, Birkus 2008, Murakami 2015}. Additionally, in vitro TAF has been shown to be efficiently taken up by hepatocytes primarily by passive permeability with a small contribution by hepatic uptake transporters OATP1B1 and OATP1B3 {Murakami 2015}. Because of the efficient uptake and intracellular metabolism, TAF resulted in intracellular concentrations of TFV-DP that are 120-fold higher compared with TFV and 5-fold higher compared with TDF

in vitro {Murakami 2015}. Thus, TAF provides enhanced delivery of TFV to the liver. In support of the concept of enhanced delivery of active drug for treatment of HIV-1 infection, a study in dogs showed approximately 70% of the orally administered TAF dose is extracted by the liver during first pass metabolism and high levels of TFV-DP were observed in the dog liver {Babusis 2013, Murakami 2015}.

Comprehensive studies have been completed characterizing the ADME profiles of TAF (and/or TFV). In addition, the potential of each agent to be involved in PK DDI has been characterized. Tabulated summaries of these results are presented in m2.6.5. The nonclinical data discussed within this document support the proposed use of TAF for the treatment of HIV-1 and chronic hepatitis B (CHB) infection. All information from nonclinical PK studies that is of relevance to the prescriber and patient has been included in the proposed prescribing information.

Nonclinical studies to characterize the absorption and disposition of TAF have been performed in mice, rats, dogs, and monkeys. The PK studies are listed in the overview table (m2.6.5, Section 1), and study details are provided in the individual study overview tables in m2.6.5. The tabulated summaries in m2.6.5 also provide TK data for studies described in m2.6.6. For the PK studies using GS-7304-02 or GS-7340-03, all doses are presented as TAF free base equivalent.

Tenofovir alafenamide was rapidly absorbed with T_{max} values of 0.08 hours in mouse and dog and 0.5 hours in monkey (the first time point assessed). Tenofovir alafenamide was undetectable in any of the rat plasma samples. Thereafter, TAF plasma concentrations declined rapidly with a terminal half-life of less than 1 hour. Concomitant with the rapid decline in TAF concentrations in plasma, the predominant metabolite TFV was formed and persisted in plasma over the dosing interval. Following oral administration of TAF to dog, high levels of TFV-DP were observed in liver and persisted with a half-life of greater than 20 hours. Furthermore, incubation of primary human hepatocytes with TAF resulted in high levels of intracellular TFV-DP in vitro.

Protein binding of TAF was moderate in human plasma with the percent unbound of 46.8% in vitro which was higher than values observed in multiple human ex vivo studies where the mean percent unbound of TAF ranged from 14% to 23% in all subjects. Following oral administration of [^{14}C]TAF to mouse, rat, and dog, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species studied. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver. High levels of radioactivity were also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. No evidence for melanin binding was observed in rats. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse. TAF poorly penetrates into cerebrospinal fluid (CSF) following oral administration in monkeys.

Metabolite profiling of TAF in mice, rats, dogs and humans demonstrated formation of purine metabolites that are also present endogenously including hypoxanthine, xanthine, allantoin, and uric acid in all species including humans. Tenofovir accounted for a majority of drug-related material in plasma, urine, and feces from all species except for human plasma in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. No metabolites unique to human were observed. Tenofovir alafenamide is not an inhibitor of

UGT1A1 and CYP enzymes except for weak inhibition observed for CYP3A in vitro. While TAF is a weak inhibitor of CYP3A in vitro, it is not a clinically meaningful inhibitor of CYP3A. Tenofovir alafenamide is not a clinically relevant inducer of CYP enzymes, UGT1A1, or P-gp. Tenofovir alafenamide was not an inhibitor of any of the transporters tested in vitro indicating that TAF is unlikely to be a perpetrator of transporter-mediated drug interactions. Tenofovir alafenamide was found to be a substrate for efflux transporters P-gp and BCRP and hepatic uptake transporters OATP1B1 and OATP1B3. A modest increase in TAF absorption has been observed upon inhibition of the intestinal efflux transporters in vitro and in vivo. Hepatic uptake transporters OATP1B1 and OATP1B3 make small contributions to TAF uptake into hepatocytes and the effects of changes in the transporter activities are not expected to be clinically relevant given the high passive permeability of TAF.

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species. The elimination of a large amount of radioactivity in bile of bile duct cannulated (BDC) dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. Total recovery of radiolabel was high for all species. Renal excretion was identified as the primary route of elimination of TFV in all species tested, and is achieved by a combination of glomerular filtration and active tubular secretion. In vitro transport studies indicate that the active tubular secretion of TFV in humans is mediated by OAT1 and multidrug resistance-associated protein (MRP)4 acting in series, as the major uptake and efflux transporters in proximal tubules, respectively. Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP1 or MRP2 are involved in the tubular efflux of TFV. While OAT1 and OAT3 transport TFV from the bloodstream into the renal proximal tubule cell, TAF is not a substrate for these transporters suggesting that TAF is not contributing to renal tubular cell loading of TFV; as a result, intracellular TFV concentrations in renal cells correlate with plasma TFV levels, which are lower following the administration of TAF than that of TDF. As the primary transporter for the tubular uptake of TFV, OAT1 has been assessed for its potential as a target for DDIs between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including COBI and protease inhibitors [PIs]). Under physiologically relevant conditions, a number of renally excreted drugs showed no effect in vitro on the OAT1-mediated transport of TFV. Similarly, PIs and COBI did not exhibit any effect on the cellular elimination of TFV mediated by the MRP4 efflux pump in vitro, indicating that PIs and COBI do not exert an effect on the accumulation of TFV in renal proximal tubules or renal elimination of TFV. Tenofovir did not inhibit the activity of the renal uptake transporter, OCT2, or the renal efflux transporter, MATE1.

B/F/TAF

No nonclinical studies have been performed assessing the metabolism of the B/F/TAF drug combination because BIC, FTC and TAF have distinct metabolic and excretion pathways for elimination. Bictegravir is metabolized by CYP3A-mediated oxidation and conjugation by UGT enzymes and then eliminated into bile, feces and urine. FTC is eliminated primarily intact by renal excretion. TAF is predominantly hydrolyzed intracellularly to TFV and is then eliminated by renal excretion. This was confirmed in a clinical DDI study (GS-US-141-1218) wherein concomitant administration of BIC and F/TAF showed no significant PK DDI and no dose adjustment was necessary when BIC was administered or coformulated together with F/TAF.

2. METHODS OF ANALYSIS

The in vivo PK, distribution, and excretion of BIC, FTC, and TAF were assessed in mouse, rat, rabbit, dog, and monkey. The in vitro absorption, metabolism, and potential for CYP or transporter mediated DDI were studied in appropriate model systems.

2.1. BIC

2.1.1. Bioanalytical Methods Supporting Pharmacokinetic Studies

2.1.1.1. Pharmacokinetic Studies

The plasma BIC concentrations in nonclinical PK studies in mouse, rat, rabbit, dog, and monkey were quantified by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) or by liquid chromatography (LC) coupled to ultraviolet (UV) detection (LC-UV) methods (m2.6.5, Section 3.1, AD-141-2307, AD-141-2279, AD-141-2286, AD-141-2296, AD-141-2306, AD-141-2300, AD-141-2280, AD-141-2281, AD-141-2284, AD-141-2297, AD-141-2282; and m2.6.5, Section 13.1.1, AD-141-2283). These methods did not strictly conform to Good Laboratory Practice (GLP) guidelines but were evaluated for selectivity, sensitivity, and linearity, as well as intra-assay accuracy and precision. The PK parameters were determined by non-compartmental analysis.

2.1.1.2. Toxicokinetic Studies

The plasma concentrations of BIC were quantified in GLP repeat-dose toxicology studies in mouse (m2.6.7, Section 7.1.1, TX-141-2042), rat (m2.6.7, Section 7.1.2, TX-141-2029 and Section 7.1.3, TX-141-2031), rabbit (m2.6.7, Section 11.1, TX-141-2035 and TX-141-2038), and monkey (m2.6.7, Section 7.1.4, TX-141-2030 and Section 7.1.5, TX-141-2032) using fully validated LC-MS/MS methods (m2.6.5, Section 2.1, BA-141-2001, BA-141-2002, BA-141-2006, BA-141-2007, and BA-141-2008). Validated parameters included selectivity, sensitivity, linearity, recovery, carryover, intra- and inter-assay precision and accuracy, sample collection stability, stock solution stability, injection medium integrity, short-term matrix stability, freeze-thaw matrix stability, long-term matrix stability, dilution integrity, and re-injection reproducibility. The lower limit of quantitation (LLOQ) for BIC was 1000 ng/mL and the upper limit of quantitation (ULOQ) for BIC was 50,000 ng/mL in mouse, rat, rabbit, and monkey plasma. The results of incurred sample reproducibility analyses in toxicology studies confirmed the repeatability of the methods. The toxicokinetic (TK) parameters were determined by non-compartmental analysis.

2.1.2. Other In Vivo Methods

Absorption, distribution, metabolism, and excretion studies were performed in mouse, rat, and monkey following a single oral dose of [^{14}C]BIC with the [^{14}C] label incorporated on the carbonyl group of the trifluorobenzyl acetamide moiety of the molecule (Table 1). Tissue distribution was determined following a single dose [^{14}C]BIC administration in non-pigmented and pigmented rats by quantitative whole body autoradiography (QWBA) and scintillation

counting (m2.6.5, Section 5.1.2 and 5.1.3, AD-141-2276). Radiocounting in the ADME study matrices were determined by liquid scintillation counting (LSC). Radiochromatograms of plasma, urine, bile, and feces were generated by LC with fraction collection followed by offline radio-detection and also characterized by LC-high resolution mass spectrometry (HRMS) (m2.6.5, Section 12.1, AD-141-2303, AD-141-2276, and AD-141-2298, and m2.6.5, Section 8.1, AD-141-2304, AD-141-2277, and AD-141-2299).

2.1.3. In Vitro Methods

2.1.3.1. Metabolism

The rate of hepatic metabolism of [^{3}H]BIC (1 μM) was assessed in hepatic microsomal fractions (1 mg/mL protein) in the presence of reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system (m2.6.5, Section 9.1.1, AD-141-2289) from nonclinical species and human.

CYP reaction phenotyping was determined by incubating [^{3}H]BIC (2 μM) with individual human recombinant CYPs (1A1, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5) co-expressed with NADPH CYP reductase (m2.6.5, Section 9.1.2, AD-141-2290). Uridine disphosphate (UDP) glucuronosyl transferase reaction phenotyping was determined by incubating BIC (5 μM) with complementary DNA (cDNA)-expressed human UGTs (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17; m2.6.5, Section 9.1.3, AD-141-2291).

Metabolism of [^{14}C]BIC was determined in cryopreserved hepatocyte preparations from human and nonclinical species (m2.6.5, Section 9.1.4, AD-141-2288).

The method of analysis was LC-MS/MS for in vitro studies with unlabeled BIC, and HPLC with radioflow detection for radiolabeled BIC.

2.1.3.2. Plasma and Microsomal Protein Binding, Blood to Plasma Ratio

The extent of BIC (2 μM) binding in plasma was assessed by equilibrium dialysis for 3 hours at 37°C (m2.6.5, Section 6.1.1, AD-141-2287). The relative protein binding of BIC (2 μM) between human plasma and cell culture medium (CCM) containing 10% fetal bovine serum was assessed in a competitive equilibrium dialysis assay at 37°C (m2.6.5, Section 6.1.1, AD-141-2287). The extent of BIC (3 μM) binding to human hepatic microsome fraction (0.5 mg protein/mL) was assessed by equilibrium dialysis at 37°C (m2.6.5, Section 6.1.2, AD-141-2311).

Blood to plasma (B/P) ratios were determined following BIC (0.5 μM) incubation in whole blood from nonclinical species and human at 37°C for 6 hours (m2.6.5, Section 5.1.1, AD-141-2312).

2.1.3.3. Permeability

The bi-directional permeability of BIC at two concentrations (10 μM and 88 μM) was assessed in human Caco-2 cell monolayers (m2.6.5, Section 3.1.1, AD-141-2295). The bi-directional permeability of BIC (10 μM) was also assessed across Madin-Darby canine kidney strain II (MDCKII) cell monolayers of wild type and in MDCKII cells overexpressing either P-gp or BCRP (m2.6.5, Section 14.1.1, AD-141-2278).

2.1.3.4. Inhibition of Cytochrome P450 enzymes and UGT1A1

The potential for BIC to reversibly inhibit the major human drug metabolizing CYP enzymes (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) and UGT1A1 was assessed using human hepatic microsomal fraction and enzyme-selective activities (m2.6.5, Section 11.1.1, AD-141-2293, and Section 11.1.2, AD-141-2294). The probe substrates for each enzyme were incubated individually with pooled human liver microsomes in the presence and absence of BIC (0 - 100 µM for CYPs; 0 – 300 µM for UGT1A1) or positive control inhibitors. The production of the enzyme-specific metabolites was measured and, where possible, the IC_{50} values were determined.

The potential for BIC mediated mechanism-based inhibition of CYPs (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A) was assessed using human hepatic microsomal fraction and enzyme-selective activities with a 2-step incubation protocol (m2.6.5, Section 11.1.3, AD-141-2308). The first stage allowed for inactivation of the enzyme in the absence of substrate, and the second stage was used to assay the remaining enzyme activity. A 10-fold dilution was performed between the 2 stages to reduce the direct inhibitory effects of the test compounds.

2.1.3.5. Induction Potential

The potential for BIC to induce metabolizing enzymes through activation of aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR) was assessed in reporter cell lines (m2.6.5, Section 11.1.4, AD-141-2292). DPX2 cells, stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase, were used for assessment of PXR activation. DRE12.6 cells, transformed with an expression vector for human AhR and the dioxin response element of the human CYP1A2 gene linked to a luciferase reporter, were used to determine AhR activation. Appropriate control compounds were also analyzed along with BIC in order to evaluate the relative induction potential of BIC.

The induction potential of BIC on CYP enzymes, UGT1A1, and P-gp was assessed in cryopreserved hepatocytes (m2.6.5, Section 11.1.5, AD-141-2305). Hepatocytes from 3 separate donors were incubated with vehicle control, appropriate positive controls, or BIC (1 – 60 µM) for a total of 3 days. Induction of CYPs (1A2, 2B6, and 3A) was measured by in situ catalytic activity assays selective for each CYP isoform. Induction of mRNA expression was determined for CYPs (1A2, 2B6, 3A4, 2C8 and 2C9), UGTs (1A1, 1A3 and 1A9) and P-gp by quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis.

2.1.3.6. Interaction with Transporters

BIC (10 µM) was assessed as a substrate for efflux transporters in transwell assays using P-gp- and BCRP-transfected MDCKII cell monolayers (m2.6.5, Section 14.1.1, AD-141-2278). Transporter expression-dependent changes in the bidirectional permeability assay were confirmed using control inhibitors. BIC (1 µM) was also evaluated as a potential substrate for uptake transporters OATP1B1 and OATP1B3 using Chinese hamster ovary (CHO) cells transfected with the individual transporters (m2.6.5, Section 14.1.2, AD-141-2275). The uptake rate of BIC in OATP1B1- and OATP1B3-overexpressing cells was determined in the absence or presence of a control inhibitor.

BIC was assessed as an inhibitor of influx and efflux using cell lines transfected with the individual transporters or membrane vesicle preparations. Inhibition of OATP1B1, OATP1B3, OAT1, OCT1, and MATE1 was studied in transfected CHO cells (m2.6.5, Section 14.1.4, AD-141-2274, Section 14.1.5, AD-141-2285, and Section 14.1.6, AD-141-2310). Inhibition of OAT3 was studied in transfected Flp-In293 cells (m2.6.5, Section 14.1.6, AD-141-2310). Inhibition of P-gp, BCRP, and OCT2 was studied in transfected MDCKII cells (m2.6.5, Section 14.1.3, AD-141-2273 and Section 14.1.5, AD-141-2285). Inhibition of BSEP was studied in cell membrane vesicles of Spodoptera frugiperda (Sf9) ovarian cells individually expressing BSEP drug transporter (m2.6.5, Section 14.1.6, AD-141-2310). Inhibition of the transport of transporter-specific probe substrates was assessed in the presence of increasing concentrations of BIC. The inhibition of the transporter-specific substrates was measured and, where possible, IC_{50} values were determined. Appropriate positive control compounds were included in each transporter assessment.

2.2. FTC

The in vivo PK, TK, distribution, and excretion of FTC were assessed in mouse, rat, and monkey. The in vitro metabolism and drug interaction characteristics of FTC were studied in appropriate model systems.

2.2.1. Bioanalytical Methods Supporting Pharmacokinetic Studies

Analytical methods used to quantify FTC in mouse, rat, and monkey plasma from the early preclinical ADME studies employed reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet detection at 280 nm. The LLOQ was 0.063 to 0.125 µg/mL {Frick 1994}. Additional mouse, rat, rabbit, and monkey PKc and TK studies used HPLC-mass spectrometry (MS)-based assays for the quantitation of FTC in plasma and urine. Initially, a method employing selected ion monitoring was developed (m2.6.5, Section 2.2, 97/001.01) and this was subsequently improved by incorporation of MS/MS detection (m2.6.5, Section 2.2, 6447v5, 7582v1, and 6159v1). Methods were cross-validated, and the LLOQ was generally in the range of 0.100 to 0.200 µg/mL.

2.2.2. Other In Vivo Methods

The recovery of radioactivity in urine and feces was determined after administration of [^{3}H]FTC to CD-1 mice (m2.6.5, Section 8.2.1, TEIN/93/0015) and samples were also subject to LC-radioprofiling. The recovery of radioactivity in feces and urine after administration of [^{14}C]FTC to Sprague-Dawley and Long Evans rats was determined (m2.6.5, Section 5.2.1, TOX092). Samples were subject to LC-radioprofiling and tissue distribution was determined by QWBA. Radioactive recovery and LC-radioprofiling studies were performed with cynomolgus monkeys after administration of [^{3}H]FTC (m2.6.5, Section 8.2.3, TEIN/93/0016) or [^{14}C]FTC (m2.6.5, Section 8.2.2, TOX063).

2.2.3. In Vitro Methods

The extent of binding of [^{3}H]FTC in plasma from mouse, rabbit, monkey, and human plasma was determined by equilibrium dialysis (m2.6.5, Section 6.2.1, TBZZ/93/0025). The potential for FTC to inhibit human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A was determined by examining its effects on selective activities catalyzed by human hepatic microsomal fraction (m2.6.5, Section 11.2.1, 15247). Positive control inhibitors were tested in parallel. The effect of FTC on glucuronidation by human hepatic microsomal fraction was also determined using 7-hydroxycoumarin as a general UGT substrate. The potential for FTC to be a substrate for human CYP enzymes was determined with 9 individual bacterially expressed CYP enzymes and through the use of human hepatic microsomal fraction and enzyme-selective inhibitors (m2.6.5, Section 9.2.1, 15396v1). Positive control substrates were tested in parallel. The role of glucuronide conjugation in the metabolism of FTC was also determined with human hepatic microsomal fraction and uridine diphosphate glucuronic acid (UDPGA) as the cofactor.

2.3. TAF

The in vivo PK, TK, distribution, and excretion of TAF were assessed in mouse, rat, dog, and monkey. The in vitro absorption, metabolism, and drug interaction characteristics of TAF were studied in appropriate model systems.

2.3.1. Bioanalytical Methods Supporting Pharmacokinetic Studies

In the early preclinical absorption studies, TFV levels in rat plasma and TAF and TFV levels in dog plasma and peripheral blood mononuclear cells (PBMCs) were determined using a fluorescence derivitization/HPLC procedure (m2.6.5, Section 3.3, R990130 and 99-DDM-1278-001-PK). Analyses of TAF and TFV in plasma and PBMCs during PK studies following single or multiple oral administration to rats (m2.6.5, Section 3.3, R2000065, AD-120-2015), mice (m2.6.5, Section 3.3, AD-120-2014 and AD-120-2016; m2.6.7, Section 6.2, TX-120-2006), dogs (m2.6.5, Section 3.3, AD-120-2034, and Section 14.3.14, AD-120-2035), and monkeys (m2.6.5, Section 3.3, P2000087) were performed using LC-MS/MS (m2.6.5, Section 2.3, BA-120-2003, BA-120-2004, BA-120-2010, BA-120-2011, BA-120-2012, and BA-120-2013). Some of these methods did not strictly conform to GLP guidelines but were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra-assay accuracy and precision.

2.3.2. Bioanalytical Methods Supporting GLP Studies

Validated methods of HPLC with MS detection were used for the earlier rat TK studies (m2.6.5, Section 2.3, 001092/NGE and R-BA-Tox-120-001). Plasma concentrations of TAF and TFV were quantified in toxicology studies in pregnant mouse, rat, and rabbit using validated LC-MS/MS methods (m2.6.5, Section 2.3, BA-120-2003, BA-120-2004, and BA-120-2005). In a 28-day toxicology study in dog, the plasma TAF concentrations were determined using a validated LC-MS/MS assay with a limit of quantification of 5 ng/mL and the concentrations of TFV in plasma and in PBMCs were determined using fluorescence derivitization followed by HPLC with fluorescence detection (m2.6.5, Section 2.4, P1278-00017) and HPLC with MS

detection (m2.6.5, Section 2.3, 993680 MYS), respectively. Analytical methods for determination of TAF in plasma and TFV in plasma and PBMCs from a 9-month dog toxicology study (m2.6.5, Section 2.3, TOX-120-002) are described within the appendices of the study report.

In a 28-day repeat dose study in monkeys, validated methods were used to determine TAF and TFV in plasma (m2.6.5, Section 2.3, 010520/PDW and 010521/PHZ) and TFV in PBMCs (m2.6.5, Section 2.3, AA01240-RQZ).

Two assay techniques were used to determine plasma TFV concentrations. The first was a reverse phase ion-pair HPLC method following fluorescence derivatization (m2.6.5, Section 2.4, P4331-00008 [mouse], P1278-00001 [rat, monkey], and P1278-00017 [dog]). This assay was validated with a LLOQ of 25 ng/mL, and was used in earlier studies. The second assay utilized LC-MS/MS and was validated with LLOQs of 1–3 ng/mL (m2.6.5, Section 2.4, P1278-00028 [rat], P1278-00029 [monkey], and P4331-0037 [dog]). The LC-MS/MS assay was also validated for the determination of TFV in rat milk with an LLOQ of 10 ng/mL (m2.6.5, Section 2.4, P1278-00034).

2.3.3. Other In Vivo Bioanalytical Methods

The ADME of TAF were assessed in various species following a single oral administration of [^{14}C]TAF. The location of [^{14}C] label at the 2- or 8-position of the adenine base is indicated by an asterisk in the structures described in Table 3. The tissue distribution of [^{14}C]TAF-derived material was assessed using QWBA of pigmented and nonpigmented male mice and rats or by LSC in dogs (m2.6.5, Section 5.3, AD-120-2009, AD-120-2011, and AD-120-2020). The metabolism and excretion of [^{14}C]TAF has been assessed in intact male mice, and in intact and BDC rats and dogs (m2.6.5, Section 8.3, AD-120-2008, AD-120-2012, and AD-120-2021). HPLC or LC-MS/MS coupled with radio flow-through detector (RFD) analysis was used for metabolite profiling and identification. Tenofovir alafenamide and its metabolites were separated using reverse phase chromatography, and detected using RFD and MS technology simultaneously. The retention times of the metabolites using reverse phase chromatography were determined by the peaks on radiochromatograms generated by an inline RFD, and the molecular ions of the metabolites were determined on the full scan mass spectra by tandem MS corresponding to the retention times of metabolites on the radiochromatograms. Tandem MS of the molecular ions was performed, and the structures of the metabolites were proposed based on their mass spectra. Where possible, the structures of the metabolites were confirmed by comparison of the chromatographic and mass spectral characteristics of the metabolites with authentic reference standards.

2.3.4. In Vitro Methods

2.3.4.1. Permeability Across Caco-2 Cell Monolayers

In vitro bidirectional permeability of TAF (incubated at 10 µM) was assessed using monolayers of the human colonic adenocarcinoma cell line Caco-2, on 12 well transwell dual chamber plates. Permeability rates were determined by quantifying the TAF levels in each chamber by LC-MS/MS (m2.6.5, Section 3.3.1, AD-120-2037). Experiments were done in the absence or in the presence of COBI, a known inhibitor of P-gp (m2.6.5, Section 14.3.3, AD-120-2013).

2.3.4.2. Stability

The stability of TAF has been assessed in plasma, intestinal S9 and hepatic S9 fractions from dog and human (m2.6.5, Section 9.3, AD-120-2025, AD-120-2024, and AD-120-2023). Intestinal stability of TAF was also studied in the presence of HIV PIs or pharmacoenhancers using intestinal S9 fractions (m2.6.5, Section 9.3.7, AD-120-2027). The disappearance of the parent prodrug was monitored by LC-MS/MS.

The stability of [^{14}C]TFV in rat hepatic microsomal fractions and in liver S9, intestinal S9, and plasma from dog and human was determined by LC-radioprofiling (m2.6.5, Section 9.3, 96-DDM-1278-003).

CYP-mediated metabolism was assessed with CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by incubating TAF at 5 µM with bacterially expressed each human CYP450 enzyme preparations (Bactosomes) coexpressed with human NADPH CYP reductase in the presence of NADPH (m2.6.5, Section 9.3, AD-120-2004). The disappearance of the parent prodrug was monitored by LC-MS/MS.

2.3.4.3. Intracellular Metabolism

Intracellular activation of TAF was assessed in primary human hepatocytes. Tenofovir alafenamide was continuously incubated with primary human hepatocytes for 24 hours and cell samples were collected at select time point followed by washing and extraction. The samples were analyzed by LC-MS/MS and intracellular concentrations of TFV-DP were quantified (m2.6.5, Section 9.3, AD-120-2017 and AD-120-2031).

2.3.4.4. Plasma Protein Binding

The binding of TAF to plasma was assessed by equilibrium dialysis against phosphate buffer at 37°C using pooled plasma from Beagle dogs and humans (m2.6.5, Section 6.3.1, AD-120-2026). Unbound TAF was quantified by LC-MS/MS.

The binding of [^{3}H]TFV in human plasma was determined by ultrafiltration (m2.6.5, Section 6.3.2, P0504-00039.1).

2.3.4.5. Inhibition of Cytochrome P450 Enzymes and UGT1A1

The inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A was assessed by incubating TAF with human liver microsomes and NADPH in the presence of individual probe substrates. Each probe substrate metabolite was quantified by LC-MS/MS. The following positive control inhibitors for each CYP isoform were used to establish the validity of the assay; α-naphthoflavone (CYP1A2), ticlopidine (CYP2B6), montelukast (CYP2C8), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4) (m2.6.5, Section 11.3.1, AD-120-2003). The mechanism-based inhibition was assessed using CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A following a 2-step incubation protocol. First, TAF or positive control inhibitors were incubated with human liver microsomes in the presence or absence of NADPH for 30 min at

37°C. The mixture was then diluted 10-fold with a phosphate buffer containing the respective probe substrate and fresh NADPH to initiate the second incubation for the CYP reaction. Each probe substrate metabolite was quantified by LC-MS/MS. The following positive control inhibitors for each CYP isoform were used to establish the validity of the assay: resveratrol and furafylline (CYP1A2); ticlopidine (CYP2B6); gemfibrozil glucuronide (CYP2C8); tienilic acid (CYP2C9); ticlopidine (CYP2C19); paroxetine (CYP2D6); and mibefradil and mifepristone (CYP3A4) (m2.6.5, Section 11.3.3, AD-120-2040).The inhibition of UGT1A1 was assessed by incubating TAF (up to 50 µM) with insect cell microsomal fraction containing expressed human UGT1A1, UDP-glucuronic acid, and a probe substrate, estradiol at 37°C. Formation of the metabolite, estradiol 3-glucuronide was monitored by LC-MS/MS (m2.6.5, Section 11.3.6, AD-120-2006).

The effects of TFV on human CYP1A2, CYP2C9, CYP2E1, CYP2D6, and CYP3A activity were determined with human hepatic microsomal fraction and enzyme-selective activities (m2.6.5, Section 11.3.2, V990172-104).

2.3.4.6. Induction Potential

The potential of TAF to induce human drug metabolizing enzymes and transporters through the activation of AhR and PXR was assessed in vitro in reporter cell lines (m2.6.5, Section 11.3.4, AD-120-2005). Briefly, assessments of induction were done using Puracyp's hepatoma-derived cell lines, DRE12.6 and DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. DRE12.6 cells are transformed with an expression vector for human AhR and the drug/dioxin response element of the human CYP1A2 gene linked to a luciferase reporter.

The induction potential of TAF on CYP activity and CYP, P-gp, and UGT1A1 mRNA levels was assessed in primary human hepatocytes (m2.6.5, Section 11.3.5, AD-120-2032). Three preparations of cryopreserved human hepatocytes isolated from 3 separate livers were treated once daily for 3 consecutive days with dimethyl sulfoxide (DMSO, 0.1% v/v, vehicle control), 1 of 3 concentrations of TAF (1, 10, or 100 µM). For positive controls for induction, omeprazole (CYP1A2) at 50 µM, phenobarbital (CYP2B6 and P-gp) at 100 µM, rifampin (CYP3A4) at 10 µM, or β-naphthoflavone (UGT1A1) at 20 µM were used. After treatment, the cells were incubated with CYP probe substrate for the analysis of phenacetin O dealkylation (marker for CYP1A2), bupropion hydroxylation (marker for CYP2B6), and testosterone 6β hydroxylation (marker for CYP3A4) by LC-MS/MS. After the activity assay, RNA was analyzed by quantitative RT-PCR (qRT PCR) to assess the effect of TAF on CYP1A2, CYP2B6, CYP3A4, P-gp, and UGT1A1 mRNA levels. In addition, a test to assess toxicity potential of TAF to human hepatocytes during the course of induction treatment was conducted with the same lots of cryopreserved human hepatocytes using the 3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

2.3.4.7. Interaction with Transporters

The potential for TAF and TFV to be a substrate for human P-gp or BCRP was assessed using monolayers of Caco-2 cells, or MDCKII cells transfected with an expression vector for the protein, and through the use of chemical inhibitors (m2.6.5, Section 14.3, AD-120-2018, AD-236-2004, AD-236-2005, and AD-104-2002). The potential for TAF and TFV to inhibit P-gp or BCRP was assessed using MDCK II cells expressing each of the transporters (m2.6.5, Section 14.3, AD-120-2019 and AD-236-2003). The potential of TAF to be a substrate or an inhibitor and the potential of TFV to be an inhibitor of human OATP1B1 and OATP1B3 were evaluated using CHO cells (m2.6.5, Section 14.3, AD-120-2019, AD-120-2022, and AD-236-2006). Interactions of FTC with human OAT1 and OAT3 (m2.6.5, Section 14.4.2, AD-236-2010) and TAF with human OAT1, OCT1, MATE1, and OCT2 were studied using CHO cells expressing the individual transporters (m2.6.5, Section 14.3.6, AD-120-2036). The effect of TAF on BSEP and OAT3 was determined using the transporter expressing Sf9 cell membrane vesicles and Flp-In 293 cells, respectively (AD-120-2036). The effects of TFV on OCT1, OCT2, MATE1, OAT1, and OAT3 were tested in CHO cells and on MRP4 and BSEP were evaluated using vesicles containing the transporter (m2.6.5, Section 14.3, AD-236-2011, AD-236-2007, AD-236-2008, and AD-104-2012).

Interactions of TFV with human MRP2 were evaluated with the human ovarian carcinoma cell line, 2008, transfected with an expression vector for the protein (m2.6.5, Section 14.3.7, AD-104-2001). Interactions of TFV with human MRP4 were performed with the human T-cell leukemic lymphoblast cells line, CEM-R1, overexpressing the protein, and were compared with the CEM-SS parental line. The potential for TFV to be a substrate for human OAT3, OCT1, or OCT2 was determined by examining uptake of [^{3}H]TFV by microinjected Xenopus laevis oocytes (m2.6.5, Section 14.3.13, PC-103-2001). The effect of TFV on human MRP1 activity was determined using MDCK II cells overexpressing the protein (m2.6.5, Section 14.3.11, PC-104-2014).

3. ABSORPTION

3.1. In Vitro Absorption Studies

3.1.1. BIC

The in vitro absorption potential of BIC was assessed by measuring the permeability across Caco-2 cell monolayers. The in vivo disposition of BIC was determined following intravenous (IV) and oral administration to Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, and rhesus monkeys. Additional oral studies were conducted in transgenic mice, Wistar Han rats, and New Zealand White (NZW) rabbits.

Permeability of BIC was studied in vitro using bidirectional permeability across Caco-2 monolayers and the results are shown in Table 4 (m2.6.5, Section 3.1.1, AD-141-2295). Bictegravir showed a dose-dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport. Overall, these data support high intestinal absorption potential for BIC in humans.

Table 4. Bidirectional Permeability of BIC in Caco-2 Monolayers

BIC Concentration (µM)	Mean P_{app} ($\times 10^{-6}$ cm/s)		Efflux Ratio
	Forward	Reverse	
10	6.2	27.2	4.4
88	14.8	22.6	1.5

Caco-2 = human colon carcinoma cell line; P_{app} = apparent permeability coefficient
Source: AD-141-2295

3.1.2. FTC

Emtricitabine shows high, dose-independent bioavailability in vivo in mice and monkeys (m2.6.5, Section 3.2); thus, in vitro absorption studies were not considered necessary.

3.1.3. TAF

Permeability of TAF was studied in both apical to basolateral (forward) and basolateral to apical (reverse) directions at 10, 100, and 1000 µM using Caco-2 monolayers (m2.6.5, Section 3.3.1, AD-120-2037). Tenofovir alafenamide showed a dose dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport (Table 5). At 100 µM TAF, the apparent forward permeability rate was 0.63×10^{-6} cm/s and the efflux ratio was 13.6. Addition of the efflux transport inhibitor, cyclosporine A (CsA) diminished the efflux ratio and increased the forward permeability.

Table 5. Dose-Dependent Bidirectional Permeability of TAF in Caco-2 Cells

Compound	Inhibitor	Concentration (µM)	Mean P_{app} ($\times 10^{-6}$ cm/s)		Efflux Ratio
			Forward	Reverse	
TAF	None	10	0.34	6.98	20.2
TAF	None	100	0.63	8.47	13.6
TAF	None	1000	1.08	5.86	6.28
TAF	10 µM CsA	10	1.51	1.34	1.00

CsA = cyclosporine A

3.2. Single Dose In Vivo Studies

3.2.1. BIC

3.2.1.1. Pharmacokinetics Following Intravenous Administration

Single-dose IV PK of BIC was determined in male rat, dog, and cynomolgus and rhesus monkeys (m2.6.5, Section 3.1, AD-141-2279, AD-141-2280, AD-141-2281, and AD-141-2282). The plasma PK parameters are summarized in Table 6.

The CL of BIC was low in rats, dogs, and monkeys (0.1% to 1.3% of hepatic blood flow). Bictegravir had a V_{ss} in animals in the range of 0.09 to 0.22 L/kg, which was lower than total body water.

Table 6. Plasma Pharmacokinetic Parameters for BIC Following a Single Intravenous Infusion Administration to Rat, Dog, and Monkey

Species	Dose[a] (mg/kg)	AUC_{inf} (nM•h)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	MRT (h)
Sprague-Dawley Rat	0.5	246000 ± 39400	0.0049 ± 0.0007	0.22 ± 0.04	32.4 ± 1.2	45.7 ± 1.7
Beagle Dog	0.5	58700 ± 17700	0.022 ± 0.006	0.15 ± 0.02	5.34 ± 0.18	7.10 ± 1.32
Cynomolgus Monkey	0.5	49400 ± 12400	0.024 ± 0.007	0.095 ± 0.010	3.58 ± 0.23	4.16 ± 0.93
Rhesus Monkey	0.5	43000 ± 5050	0.026 ± 0.003	0.11 ± 0.02	3.76 ± 0.76	4.36 ± 1.30

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; MRT = mean residence time; $t_{1/2}$ = estimated plasma elimination half-life; V_{ss} = volume of distribution at steady state
a Formulated as solution in 5% ethanol, 55% PEG 300, and 40% water.
Values are the mean ± standard deviation from 3 animals.
BIC: 1 nM = 0.449 ng/mL
Source: AD-141-2279, AD-141-2280, AD-141-2281, and AD-141-2282

3.2.1.2. Pharmacokinetics Following Single Dose Oral Administration

The PK of BIC was determined in male rat, dog, and monkey following administration of oral solutions (m2.6.5, Section 3.1, AD-141-2279, AD-141-2280, and AD-141-2281). The plasma PK parameters are summarized in Table 7. Bictegravir was absorbed quickly following oral solution administration, reaching maximal plasma concentrations (C_{max}) within 4 hours postdose. The oral

bioavailability of BIC solution formulation was moderate to high (42% to 74%). The high forward permeability of BIC in Caco-2 cells and the high bioavailability of BIC in monkeys were consistent with high intestinal absorption in humans.

Table 7. Plasma Pharmacokinetic Parameters Following a Single Oral Administration of BIC in Solution to Rat, Dog and Monkey

Species	Dose[a] (mg/kg)	AUC_{inf} (nM•h)	C_{max} (nM)	T_{max} (h)	$t_{1/2}$ (h)	F (%)
Sprague-Dawley Rat	0.5	125000 ± 43000	3480 ± 773	4.00 ± 2.00	25.7 ± 1.9	49.8 ± 16.8
Beagle Dog	1.0	55900 ± 18500	9720 ± 1130	0.83 ± 0.29	4.26 ± 0.40	41.8 ± 13.9
Cynomolgus Monkey	1.0	72500 ± 39500	16600 ± 4540	0.83 ± 0.29	3.26 ± 0.50	73.8 ± 40.3

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; F = oral bioavailability; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration
a Formulated as a solution - 5% ethanol, 55% PEG 300, and 40% water for rat and 30% Captisol in water for dog and monkey. Values are the mean ± standard deviation from 3 animals.
BIC: 1 nM = 0.449 ng/mL
Source: AD-141-2279, AD-141-2280, and AD-141-2281

The PK of BIC was also determined in mouse, rat, rabbit, and monkey following single increasing oral doses to inform dose and formulation selection for repeat dose toxicology studies (m2.6.5, Section 3.1, AD-141-2307, AD-141-2286, AD-141-2306, AD-141-2296, AD-141-2300, and AD-141-2297).

3.2.1.2.1. Mouse

The oral PK of BIC was determined over a dose range of 30 to 1500 mg/kg in the transgenic mouse (model 001178-W [wild type] RasH2; Table 8). The plasma exposure (AUC and C_{max}) of BIC increased with dose in the range of 30 to 1000 mg/kg; the increase in exposure was less than dose proportional. At 1000 mg/kg, exposure to BIC plateaued, with a decrease in exposure noted at the higher dose of 1500 mg/kg in both males and female mice. No gender differences in AUC or C_{max} were observed.

Table 8. Plasma Pharmacokinetic Parameters for BIC Following Single Oral Administration to Transgenic (wild type) RasH2 Mouse[a]

Dose[b] (mg/kg)	AUC_{0-24h} (µg•h/mL)		C_{max} (µg/mL)		T_{max} (h)		C_{24h} (µg/mL)	
	Male	Female	Male	Female	Male	Female	Male	Female
30	660 ± 34	745 ± 37	59.3 ± 22.2	71.8 ± 7.3	0.5	2.0	5.88 ± 0.90	5.66 ± 1.48
100	1257 ± 53	1509 ± 141	97.9 ± 16.9	108 ± 16	1.0	8.0	8.98 ± 1.89	11.1 ± 3.8
300	2106 ± 107	2173 ± 232	116 ± 10	127 ± 15	4.0	8.0	24.2 ± 6.2	20.2 ± 2.4
1000	2568 ± 100	3197 ± 301	135 ± 7	163 ± 13	0.5	2.0	43.3 ± 19.8	57.9 ± 15.7
1500	2155 ± 110	2366 ± 143	123 ± 6	164 ± 2	4.0	2.0	45.3 ± 28.3	37.4 ± 36.6

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; T_{max} = time to reach the maximum plasma concentration.
a Transgenic mouse model 001178-tg/wt [CByB6F1-Tg(HRAS)2Jic]
b Formulated as a suspension in 0.5% HPMC K100LV and 0.1% Tween 20 in water.
BIC: 1 nM = 0.449 ng/mL
Source: AD-141-2307

3.2.1.2.2. Rat

The oral PK of BIC (free acid and the sodium salt) was determined over a wide dose range in the male Wister Han rat. Aqueous suspension and organic solution formulation were compared using BIC free acid to identify a formulation that provided optimal exposure for use in repeat-dose toxicology studies and the results are summarized in Table 9. At low doses (10 and 30 mg/kg), the organic vehicle provided higher plasma exposures (AUC and C_{max}) of BIC compared to the aqueous suspension. At 100 mg/kg, exposure to BIC plateaued with the organic vehicle, with a decrease in exposure noted at the higher dose of 300 mg/kg. Similarly, exposure to BIC plateaued at 300 mg/kg with the aqueous vehicle with a decrease noted at 1000 mg/kg. Maximal exposures were similar with the aqueous and organic vehicles. Thus the aqueous suspension was chosen for use in single dose safety pharmacology and repeat dose toxicity studies in rats. The PK following oral dosing of BIC sodium salt, the form used in pivotal repeat-dose toxicology studies, is also summarized in Table 9. The C_{max} and AUC were comparable between the free acid and sodium salt form of BIC.

Table 9. Plasma Pharmacokinetic Parameters for BIC Following Single Oral Administration to Male Wistar Han Rat

	AUC_{0-24h} (µg•h/mL)			**C_{max} (µg/mL)**		
Dose (mg/kg)	**Organic Vehicle[a] (Free Acid)**	**Aqueous Suspension[b] (Free Acid)**	**Aqueous Suspension[b] (Sodium Salt)**	**Organic Vehicle[a] (Free Acid)**	**Aqueous Suspension[b] (Free Acid)**	**Aqueous Suspension[b] (Sodium Salt)**
10	929 ± 97	471 ± 142	-	61.5 ± 2.1	31.1 ± 6.0	-
30	1904 ± 249	849 ± 66	926 ± 209	114 ± 5	54.3 ± 5.8	55.3 ± 6.0
100	2847 ± 229	1625 ± 826	1896 ± 331	148 ± 7	104 ± 30	102 ± 17
300	2137 ± 570	2205 ± 248	2436 ± 481	105 ± 26	120 ± 23	129 ± 9
1000	-	1931 ± 109	-	-	115 ± 14	-

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; C_{max} = maximum plasma concentration
a Formulated as a solution in 10% ethanol, 10% propylene glycol, 40% Labrasol, and 40% Solutol® HS 15.
b Formulated as a suspension in 0.5% HPMC K100LV and 0.1% Tween 20 in water.
Values are the mean ± standard deviation from 3 animals.
1 nM BIC = 0.449 ng/mL
Source: AD-141-2306, AD-141-2286 and AD-141-2296

3.2.1.2.3. Rabbit

The oral PK of BIC over a dose range from 100 to 1000 mg/kg was determined in the NZW rabbit and the results are summarized in Table 10. The systemic plasma exposure (AUC and C_{max}) of BIC administered as an aqueous suspension increased with dose level from 100 to 1000 mg/kg. The increase in AUC exposure was dose proportional from 100 to 300 mg/kg, but less than dose proportional from 300 to 1000 mg/kg.

Table 10. Plasma Pharmacokinetic Parameters for BIC (Sodium Salt) Following Single Oral Administration to Female NZW Rabbits

Dose[a] (mg/kg)	AUC_{0-24h} (µg•h/mL)	C_{max} (µg/mL)	T_{max} (h)	C_{24h} (µg/mL)
100	23.3 ± 1.9	4.38 ± 0.21	1.67 ± 0.58	0.32 ± 0.12
300	69.7 ± 6.9	6.41 ± 1.73	2.00 ± 0.00	1.82 ± 0.37
1000	171 ± 64	9.76 ± 3.49	16.7 ± 12.7	9.29 ± 4.30

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; T_{max} = time to reach the maximum plasma concentration.
a Formulated as a suspension in 0.5% HPMC K100LV and 0.5% Tween 20 in water.
Values are the mean ± standard deviation from 3 animals.
1 nM BIC = 0.449 ng/mL
Source: AD-141-2300

3.2.1.2.4. Monkey

The oral PK of BIC was determined in the cynomolgus monkey with two forms of BIC (free acid and the sodium salt) at doses up to 1000 mg/kg and the results are summarized in m2.6.5, Section 3.1. The plasma exposures were similar between the free acid and the sodium salt forms. The PK following oral dosing of the BIC sodium salt, the form selected for pivotal toxicology studies, is summarized in Table 11. The plasma exposure (AUC and C_{max}) of BIC increased with dose in the range of 30 to 1000 mg/kg; the increase was less than dose proportional.

Table 11. Plasma Pharmacokinetic Parameters for BIC (Sodium Salt) in Male Cynomolgus Monkeys Following Single Ascending Oral Doses in Aqueous Suspension

Dose[a] (mg/kg)	AUC_{0-24h} (µg•h/mL)	C_{max} (µg/mL)	T_{max} (h)	C_{24h} (µg/mL)
30	171 ± 73	18.7 ± 4.0	2.67 ± 1.15	2.32 ± 1.93
100	348 ± 51	42.1 ± 10.3	2.67 ± 1.15	3.42 ± 1.21
1000	1056 ± 339	80.9 ± 25.6	5.33 ± 1.15	13.4 ± 3.6

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; T_{max} = time to reach the maximum plasma concentration.
a Formulation contained 0.5% HPMC K100LV and 0.1% Tween 20 in water.
Values are the mean ± standard deviation from 3 animals.
BIC: 1 nM = 0.449 ng/mL
Source: AD-141-2297

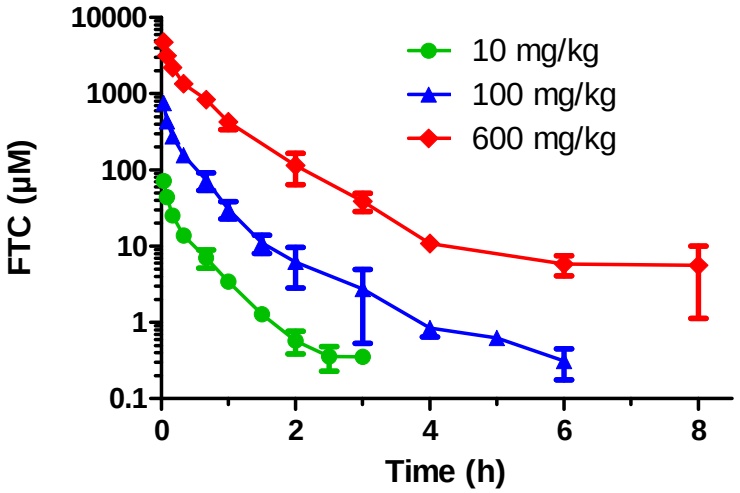
3.2.2. FTC

3.2.2.1. Mouse

The FTC PK profile was determined after IV and oral administration to nonfasted male CD-1 mice at doses of 10 mg/kg (m2.6.5, Section 3.2.1, TEIN/93/0003), 100 mg/kg (m2.6.5, Section 3.2.2, TEIN/93/0004), and 600 mg/kg (m2.6.5, Section 3.2.3, IUW00101). Pharmacokinetic profiles are illustrated in Figure 1 and Figure 2. After IV administration, the decline in plasma concentration was bi- or tri-exponential, with V_{ss} values close to that of total

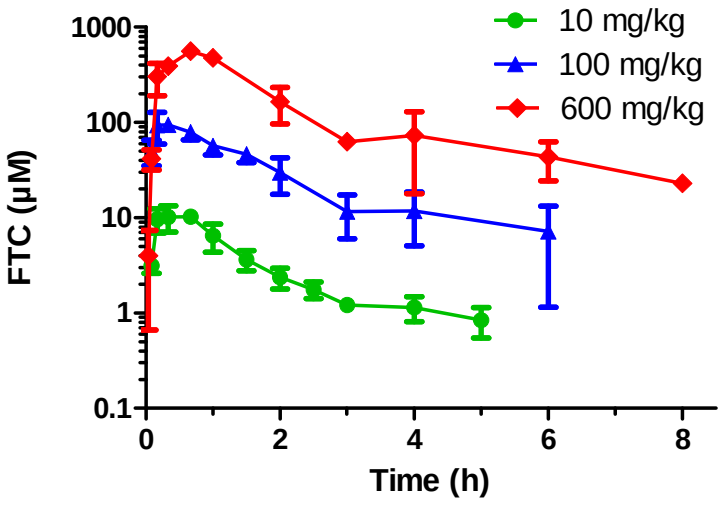
body water (0.89–1.1 L/kg) and clearance values (1.28–2.33 L/h/kg) exceeding the glomerular filtration rate in mice. After oral administration, absorption was rapid and extensive, with absolute bioavailability values of 96%, 79%, and 63% at 10, 100, and 600 mg/kg, respectively. C_{max} and AUC values increased roughly dose-proportionally from 10 to 600 mg/kg.

Figure 1. **Mean Plasma Concentration vs Time Profile Following an Intravenous Bolus Dose of FTC in Solution to Male CD-1 Mice (mean ± SD)**



FTC = emtricitabine; SD = standard deviation
Source: Reports TEIN/93/0003 (10 mg/kg, n=5), TEIN/93/0004 (100 mg/kg, n=5) and IUW00101 (100 mg/kg, n=3)

Figure 2. **Mean Plasma Concentration vs Time Profile Following an Oral Dose of FTC in Solution to Male CD-1 Mice (mean ± SD)**

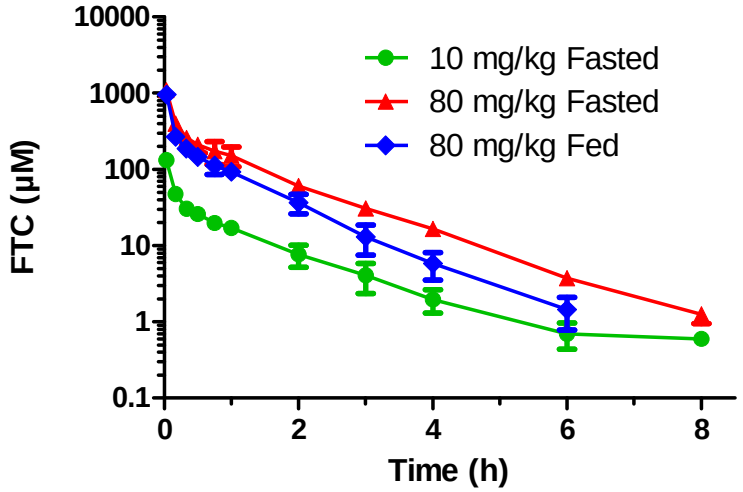


FTC = emtricitabine; SD = standard deviation
Source: Reports TEIN/93/0003 (10 mg/kg, n=5), TEIN/93/0004 (100 mg/kg, n=5) and IUW00101 (100 mg/kg, n=3)

3.2.2.2. Monkey

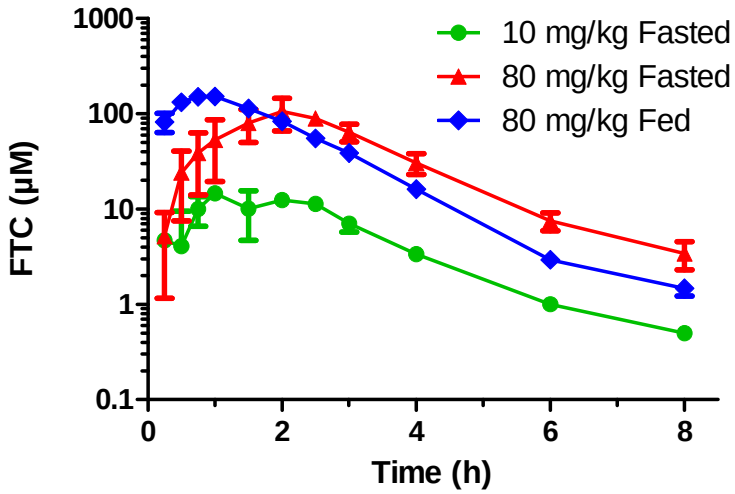
Two studies were performed with male cynomolgus monkeys dosed with a solution of FTC orally or by slow (2-minute) IV bolus administration. In the first study, 8 monkeys were administered single 10 mg/kg or 80 mg/kg doses of FTC (4 monkeys at each dose) in a crossover design study (m2.6.5, Section 3.2.4, TEZZ/93/0019). Animals were fasted overnight to 2 hours postdose. In a second study (m2.6.5, Section 3.2.5, IUW00301), 4 nonfasted monkeys were administered 80 mg/kg FTC intravenously or orally in a crossover design. Pharmacokinetic profiles are illustrated in Figure 3 and Figure 4. Pharmacokinetic parameters were similar for all 3 IV dose groups with V_{ss} values similar to those for total body water (0.77–0.80 L/kg) and moderate clearance values (0.70–0.97 L/kg). Half-lives were independent of dose and route of administration. In the fasted state, absorption was rapid and oral bioavailability values were 62.7% and 57.5% at 10 and 80 mg/kg, respectively. In the fed state, absorption was slower and the oral bioavailability was 97.4% at 80 mg/kg.

Figure 3. Mean Plasma Concentration vs Time Profile Following an Intravenous Bolus Dose of FTC in Solution to Male Cynomolgus Monkeys (mean ± SD, n = 4)



FTC = emtricitabine; SD = standard deviation
Source: Reports TEZZ/93/0019 (fasted animals) and IUW00301 (fed animals)

Figure 4. **Mean Plasma Concentration vs Time Profile Following an Oral Dose of FTC in Solution to Male Cynomolgus Monkeys (mean ± SD, n = 4)**



FTC = emtricitabine; SD = standard deviation
Source: Reports TEZZ/93/0019 (fasted animals) and IUW00301 (fed animals)

3.2.3. TAF and TFV

3.2.3.1. Mouse

The mouse plasma PK studies were conducted by dosing either GS-7340-02 or GS-7340-03 to male CD-1 mice, or GS-7340-03 to both male and female 001178-W mice (m2.6.5, Section 3.3.2, AD-120-2014 and Section 3.3.3, AD-120-2016). Although TAF was observed in mouse plasma in a dose dependent manner, the concentrations were limited and $t_{1/2}$ could not be determined. Tenofovir exposure increased with the increase in dose and was greater than dose proportional between 10 to 100 mg/kg. Gender differences in plasma TFV levels were less than 2-fold in C_{max} and AUC_{0-t} values. The PK profiles for the 2 different fumarate forms were generally similar. Pharmacokinetic parameters for GS-7340-02 and GS-7340-03 are summarized in Table 12. Values for C_{max} and AUC_{0-t} for TFV were generally similar between GS-7340-02 and GS-7340-03. Together with the rat PK results, the 2 different forms of TAF do not affect the pharmacokinetic properties. The PK parameters following a single dose of GS-7340-03 to 001178-W wild type mice are summarized in Table 13. Measurable concentrations were limited for TAF. No consistent gender-based differences were observed in TAF C_{max} and AUC_{0-t} values. Tenofovir alafenamide was extensively converted to TFV in 001178-W mice following oral gavage administration of GS-7340-03. Exposure to TFV increased with the increase in dose level from 10 to100 mg/kg. The increases in C_{max} and AUC_{0-t} were greater than dose proportional between the 10 to 100 mg/kg. Gender differences in plasma concentration of TFV were less than 2-fold in C_{max} and AUC_{0-t} values.

Table 12. Dose Dependent Plasma Pharmacokinetic Parameters Following a Single Oral Administration of GS-7340-02 and GS-7340-03 to Male CD-1 Mice

Test Article	GS-7340-02						GS-7340-03					
Dose (mg/kg)	10		30		100		10		30		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
C_{max} (µg/mL)	5.53	106	NA	440	37.1	1827	NA	85.4	10.3	383	34.7	2152
T_{max} (h)	0.083	0.50	NA	0.25	0.083	0.75	NA	0.50	4.00	0.50	0.25	1.50
$t_{1/2}$ (h)	NA	NA	NA	NA	NA	NA	NA	5.16	NA	10.1	NA	NA
AUC_{0-t} (ng•h/mL)	NA	455	NA	2005	26.0	10643	NA	493	NA	2477	11.3	10866

NA = not applicable
Source: AD-120-2014

Table 13. Dose Dependent Plasma Pharmacokinetic Parameters Following a Single Oral Administration of GS-7340-03 to 001178-W Wild Type Mice

Dose (mg/kg)	10				30				100			
Analyte	TAF		TFV		TAF		TFV		TAF		TFV	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
C_{max} (ng/mL)	NA	NA	175	100	8.80	117	615	421	648	280	1988	1733
T_{max} (h)	NA	NA	0.25	0.50	0.083	0.5	0.25	0.25	0.25	0.50	0.50	0.50
$t_{1/2}$ (h)	NA	NA	9.78	8.20	NA	NA	9.51	10.9	NA	NA	8.04	11.0
AUC_{0-t} (ng•h/mL)	NA	NA	735	354	NA	NA	2639	2053	194	104	10026	7131

NA = not applicable
Source: AD-120-2016

3.2.3.2. Rat

Plasma PK studies following a single oral administration of TAF were performed to determine PK parameters for TFV, to assess potential differences between GS-7340-02 and GS-7340-03, and to compare exposure to TFV between TAF and TDF (m2.6.5, Section 3.3, R990130, AD-120-2015, and R2000065). In all cases TAF was rapidly absorbed and converted to TFV. The plasma TFV T_{max} was less than 1 hour for all doses tested. Tenofovir exposure increased with the increase in dose and was greater than dose proportional between 5 to 100 mg/kg. No significant differences in PK profiles were observed between GS-7340-02 and GS-7340-03 (Table 14). The plasma C_{max} and AUC for TFV were 2- to 3-fold higher when Sprague-Dawley rats were orally dosed with 400 mg/kg TAF compared to 400 mg/kg TDF (R2000065).

Table 14. Dose Dependent Plasma Pharmacokinetic Parameters following a Single Oral Administration of GS-7340-02 and GS-7340-03 to Male Sprague-Dawley Rats

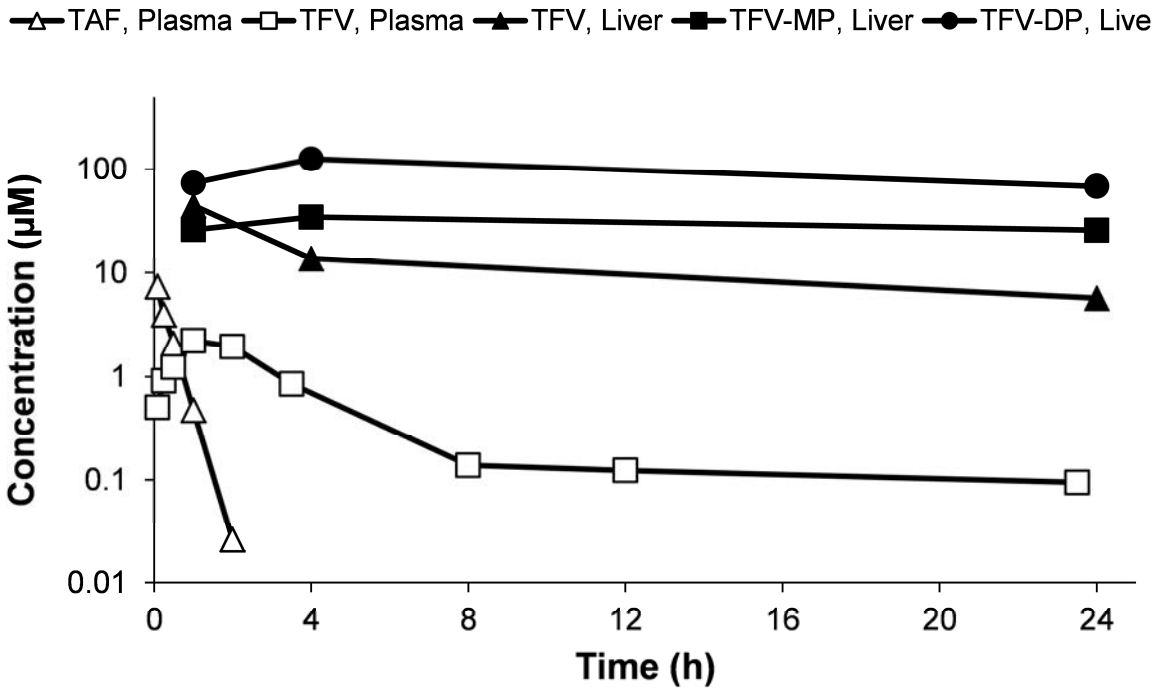
Test Article	GS-7340-02			GS-7340-03		
Dose (mg/kg)	**5**	**25**	**100**	**5**	**25**	**100**
Analyte	**TFV**	**TFV**	**TFV**	**TFV**	**TFV**	**TFV**
C_{max} (µg/mL)	32.5	199	1240	39.3	364	1670
T_{max} (h)	0.667	0.583	0.833	0.583	0.833	0.667
$t_{1/2}$ (h)	NA	11.2	10.3	NA	7.89	7.85
AUC_{0-t} (ng•h/mL)	122	1395	7771	88.5	1810	9759

3.2.3.3. Dog

In order to assess the effects of the stereo configuration, fumarate form, food, and the route of administration, the plasma and PBMC PK profiles were determined in Beagle dogs following IV bolus (GS-7340-02 [6.3 mg/kg]), or oral administration (TAF as free base [18.0 mg/kg], its diastereomer GS-7339 [18.0 mg/kg], the mixture GS-7171 [16.0 mg/kg], or GS-7340-02 [4,8, 5.0, and 20 mg/kg under fasted and 5.0 mg/kg under fed conditions]) (m2.6.5, Section 3.3.7, 99-DDM-1278-001-PK). Following oral administration, TAF and its diasteroisomer were rapidly absorbed and eliminated with a T_{max} of less than 0.5 h and $t_{1/2}$ ranging from 0.2 hours to 0.9 hours. The plasma exposures to the intact prodrugs were similar when TAF or GS-7339 were dosed separately, however, when the isomeric mixture, GS-7171, was dosed, the exposure to GS-7339 was approximately 3-fold higher than TAF. The TFV exposure in plasma was similar for both diastereomers. The TFV exposure in PBMCs was approximately 4-fold higher when animals were dosed with TAF than GS-7339. All the PK parameters were similar for the free base and fumarate form. Plasma exposures to TAF and TFV and PBMC exposure to TFV were approximately 2.5-fold higher in fasted dogs than in fed dogs.

The plasma and liver PK profiles were determined following a single oral dose of 10 mg/kg TAF to male Beagle dogs (m2.6.5, Section 3.3.8, AD-120-2034). Tenofovir alafenamide was rapidly absorbed and eliminated with observed plasma T_{max} of 0.08 hours and $t_{1/2}$ of 0.24 hours. Tenofovir was the major metabolite found in plasma with a C_{max} value of 2.23 µM. The pharmacologically active metabolite, TFV-DP was the major metabolite in liver achieving a C_{max} of 126 µM at 4.0 hours postdose. The plasma and liver PK profiles are summarized in Figure 5.

Figure 5. Plasma and Liver PK following a single dose of TAF to Beagle Dogs



3.2.3.4. Monkey

The plasma PK profiles for TAF and TFV as well as TFV concentrations in PBMCs were determined in rhesus monkeys following a single oral dose of GS-7340-02 at 0.5, 5.0, and 50 mg/kg (m2.6.5, Section 3.3.9, P2000087). The plasma PK parameters are summarized in Table 15. Tenofovir alafenamide and TFV levels increased rapidly with T_{max} values of approximately 0.5 and 1 hour, respectively. The TFV levels in PBMCs were determined before and after treatment with acid phosphatase, which was used to convert the phosphorylated metabolites to TFV (Table 16). Tenofovir persisted in PBMCs up to 96 hours with an apparently slower decline in PBMCs than in plasma. The TFV levels were significantly higher in the samples treated with acid phosphatase suggesting that a significant proportion of TFV-related material in PBMCs was in phosphorylated forms.

Table 15. Dose Dependent Plasma Pharmacokinetic Parameters for TAF and TFV Following a Single Oral Administration of GS-7340-02 to Rhesus Monkeys

GS-7340-02 Dose (mg/kg)	0.5	5	50	0.5	5	50
Analyte	**TAF**			**TFV**		
C_{max} (ng/mL)	2.79	125	4143	7.72	161	1326
T_{max} (h)	0.38	0.8	0.5	1	1.33	1.0
$t_{1/2}$ (h)	0.61	0.23	0.40	4.62	9.92	17.33
$AUC_{0\text{-last}}$ (ng·h/mL)	1.22	95.1	3811	39.9	1037	9934
$AUC_{0\text{-}\infty}$ (ng·h/mL)	2.47	80.0	3846	52.7	1069	10250

Table 16. Concentrations of TFV in PBMCs from Monkeys Following a Single Oral Administration of GS-7340-02 at 5 and 50 mg/kg

	TFV PBMC Levels (ng/10^6 Cells)			
	Without Phosphatase Treatment		With Phosphatase Treatment	
GS-7340-02 Dose (mg/kg)	5	50	5	50
2 h	0.47	17.0	0.73	34.2
24 h	0.06	6.82	0.62	20.1
96 h	BLQ	3.03	0.18	8.68

BLQ = below the limit of quantitation

3.3. Repeat-Dose In Vivo Studies

3.3.1. BIC

The TK profiles of BIC following repeat dose oral administration were examined in mouse (m2.6.7, Section 7.1.1, TX-141-2042), rat (m2.6.7, Section 7.1.2, TX-141-2029 and Section 7.1.3, TX-141-2031), rabbit (m2.6.7, Section 11.1, TX-141-2035 and TX-141-2038), and monkey (m2.6.7, Section 7.1.4, TX-141-2030 and Section 7.1.5, TX-141-2032) as part of toxicology studies. The results of these studies are detailed in Section 3.1 of m2.6.6. Bictegravir plasma exposure increased following repeat oral administration of BIC; the increases were less than dose proportional. In rats, females had 2-to 3-fold higher BIC exposures than males at the high 300 mg/kg/day dose. None to low accumulation (up to 3-fold) of BIC was observed in rats after repeat dosing. In cynomolgus monkeys, gender-based differences were less than 2-fold in BIC exposures and no accumulation (< 2-fold) of BIC was observed after repeat dosing.

3.3.2. FTC

Multiple dose in vivo TK studies were performed in mouse, rat, and monkey in support of safety evaluation. The results are presented in detail in m2.6.7, Section 3.2 and general conclusions from representative studies are noted below.

3.3.2.1. Mouse

Toxicokinetic data have been generated from a number of short- to long-term studies in mice, following 14-day to 6-month dosing of FTC with doses ranging from 0 to 3000 mg/kg/day. The systemic exposure of FTC at steady state increased proportionally with dose administered (m2.6.7, Section 7.2, TOX001, TOX599, TOX022 [and TOX022 PK report], TOX628).

In a 2-year oral oncogenicity study, FTC was administered once daily to CD-1 mice by oral gavage at doses of 0, 80, 250, and 750 mg/kg/day (m2.6.5, Section 4.2.1, TOX-109). Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring 0.5 to 1.0 hour postdose. C_{max} and $AUC_{0–24}$ results are summarized in (TOX-109). $AUC_{0–24}$ and C_{max} increased proportionally with dose over the range of 80 to 750 mg/kg/day. In general, the exposures ($AUC_{0–24}$) in male mice were similar to those in female mice at all doses. $AUC_{0–24}$ and C_{max} values were higher on Week 26 compared to Week 2.

3.3.2.2. Rat

Toxicokinetic data from a subchronic study in rats with FTC doses showed linear relationship between systemic exposure and daily dose of FTC from 120 to 3000 mg/kg (m2.6.7, Section 7.2.5, TOX097).

In a 2-year oral oncogenicity study, FTC was administered once daily to Sprague-Dawley rats by oral gavage at doses of 0, 60, 200, and 600 mg/kg/day. Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring at 0.5 hours postdose. C_{max} and $AUC_{0–24}$ results are summarized in m2.6.5, Section 4.2.2, TOX-108. AUC_{0-24} and C_{max} increased with dose over the range of 60 to 600 mg/kg/day. In general, exposure ($AUC_{0–24}$) in male rats was similar to those in female rats. $AUC_{0–24}$ and C_{max} values were higher on Week 26 compared to Week 2.

3.3.2.3. Monkey

A 1-month toxicology study of FTC was conducted in cynomolgus monkeys at oral doses of 0, 80, 400, and 2000 mg/kg/day, given in 2 divided doses, 6 hours apart (m2.6.7, Section 7.2.6, TOX600). Plasma concentrations of FTC were measured in samples drawn predose and over the first 6 hours after the first dose on Days 3 and 27. Cerebrospinal fluid and corresponding plasma samples were obtained for analysis at 1 hour postdose on Day 28. There were no significant differences in drug levels in plasma and CSF between males and females. No significant differences in PK parameters were determined between Day 3 and Day 28. Mean C_{max} values increased with dose and AUC_{0-6} were proportional to the dose. The overall mean (combined male, female and dose day) C_{max} values were 13.9, 62.8, and 198 µg·h/mL for monkeys given 40, 200, and 1000 mg/kg/dose, respectively. The overall AUC_{0-6} were 13.9, 62.8, and 198 µg·h/mL for monkeys given 40, 200, and 1000 mg/kg/dose, respectively. The concentration of FTC in CSF 1 hour after dosing on Day 28 averaged 3.9 ± 0.7 percent of the corresponding plasma levels.

A 3-month oral toxicity study was performed with FTC in cynomolgus monkeys (m2.6.7, Section 7.2.7, TOX627). The doses tested were 0, 40, 200, and 1000 mg/kg/day (n = 5/sex/group), given as 2 divided doses by nasogastric intubation, with approximately 6 hours between doses. Emtricitabine was rapidly and well absorbed with peak plasma concentrations occurring between 0 and 2 hours. No significant differences were seen between results from males and females at any dose level and there were no significant changes in PK parameters between dose Days 3 and 87. Maximum plasma levels of FTC increased with dose, but increased linearly only between the 40 and 200 mg/kg/day doses. The overall mean (combined male, female and dose day) C_{max} values were 5.63, 25.2, and 101 µg·h/mL for monkeys given 40, 200, and 100 mg/kg/day, respectively. The AUC_{0-6} values were proportional to the dose. The overall AUCs were 13.3, 60.8, and 310 µg·h/mL for monkeys given 40, 200, and 1000 mg/kg/day, respectively. Similar AUCs across multiple days suggest that there was no significant accumulation of FTC over the dosing period.

A 1-year oral toxicity study was performed with FTC in cynomolgus monkeys (m2.6.7, Section 7.2.8, TOX032). The doses tested were 0, 50, 200, and 500 mg/kg/day, each given as 2 divided doses by nasogastric intubation, with approximately 5 hours between doses. Emtricitabine was rapidly and well absorbed with peak plasma concentrations occurring between 0.5 to 2 hours after dosing. Plasma FTC was eliminated with a terminal $t_{1/2}$ of 2 to 4 hours at all dose levels. The $t_{1/2}$ estimates did not change after multiple-dose administration. There were no major differences in plasma FTC exposure between male and female monkeys. Slightly higher plasma exposures to FTC were achieved at Weeks 13, 26, and 52 as compared to Day 0 for each dose level. Plasma C_{max}, $AUC_{0–6}$, and estimated steady-state $AUC_{0–24}$ (Weeks 13, 26, and 52) increased linearly with the dose administered over the range of 50 to 500 mg/kg/day in both male and female monkeys.

3.3.3. TAF

3.3.3.1. Mouse

GS-7340-02 was administered by oral gavage for up to 14 days to male and female Crl:CD1(ICR) mice at a dose of 100, 500, or 1000 mg/kg/day (m2.6.7, Section 6.2, TX-120-2006). Due to early death for animals given 500 or 1000 mg/kg/day, only the 100 mg/kg/day dose group was evaluated. GS-7340 at 100 mg/kg/day corresponded to a Day 14 C_{max} of 27.1 and 2.89 ng/mL for males and females, respectively; the AUC_{0-24} could not be calculated due to the lack of a distinct elimination phase. GS-7340 rapidly converted to its metabolite TFV. There were no significant differences in TFV PK profiles between males and females.

Following daily administration of GS-7340-02 to mice via oral gavage for at least 13 weeks at doses of 0, 10, 30, and 100 mg/kg/day, the PK parameters for TAF and TFV were determined (m2.6.7, Section 7.3.1, TX-120-2007). Blood samples were collected from up to 3 TK animals/sex/group/time point on Day 1 and during Week 13 predose and at approximately 0.25, 0.5, 1, 4, 8, and 24 hours postdose. Exposure to TFV increased with the increase in GS-7340-02 dose from 10 to 100 mg/kg/day. The increases in C_{max} and AUC_{0-t} were generally greater than proportional between the 10 to 100 mg/kg/day dose levels. Gender-based differences were less than 2-fold in TFV C_{max} and AUC_{0-t} values. No unexpected accumulation of TFV was observed after multiple dosing. Tenofovir alafenamide was rapidly and extensively converted to TFV after oral administration in mice.

3.3.3.2. Rat

The plasma PK profile of TFV was determined during the course of a 28 day oral gavage toxicity study in adult male and female albino (Sprague-Dawley) rats following daily administration of either 1.5, 6.25, 25, 100 or 400 mg/kg/day of GS-7340-02 (m2.6.7, Section 7.3.2, R990182). A nonlinear PK response was observed for total plasma exposure versus dose for both sexes on both Day 1 and Day 28. A greater than linear increase in plasma exposure was observed as the dose was increased. There was no observed plasma accumulation (accumulation ratio 0.96 to 1.11) over the 28-day study for any dose group.

In a 26-week toxicology study, GS-7340-02 was administered once daily at doses of 0 (vehicle only), 5, 25 and 100 mg/kg/day by oral gavage and plasma PK parameters of TFV were determined on Day 1 and during Weeks 13 and 26 (m2.6.7, Section 7.3.3, TOX-120-001). No consistent differences in plasma PK parameters were found between male and female rats. Mean TFV C_{max} and AUC values increased dose proportionally over the dose range of 5 to 100 mg/kg/day. Mean TFV AUC obtained on Day 1 was slightly lower than that measured during Weeks 13 and 26, which suggested that there was a slight accumulation of TFV with repeat dosing.

3.3.3.3. Dog

Following daily oral administration of 8.29 mg/kg TAF for 7 days to male Beagle dogs, the plasma and liver PK profiles were determined on Day 1 and 7 (m2.6.5, Section 4.3.1, AD-120-2033). As shown in Figure 6, TAF was rapidly absorbed and exhibited a short terminal half-life ($t_{1/2}$) of 0.3 hours in plasma on both Day 1 and 7. The rapid disappearance of TAF was accompanied by an increase in TFV. Tenofovir was the major metabolite detected in plasma achieving a maximal plasma concentration (C_{max}) of 1.47 and 2.12 µM on Day 1 and 7, respectively. The pharmacologically active diphosphate metabolite, TFV-DP, was efficiently formed in dog livers achieving concentrations of 242 and 153 µM at 4.0 and 24 hours postdose on Day 7, respectively (Figure 7).

Figure 6. Plasma PK of TAF and TFV on Day 1 and Day 7 Following Repeat Dose Oral Administration of TAF at 8.29 mg/kg/day

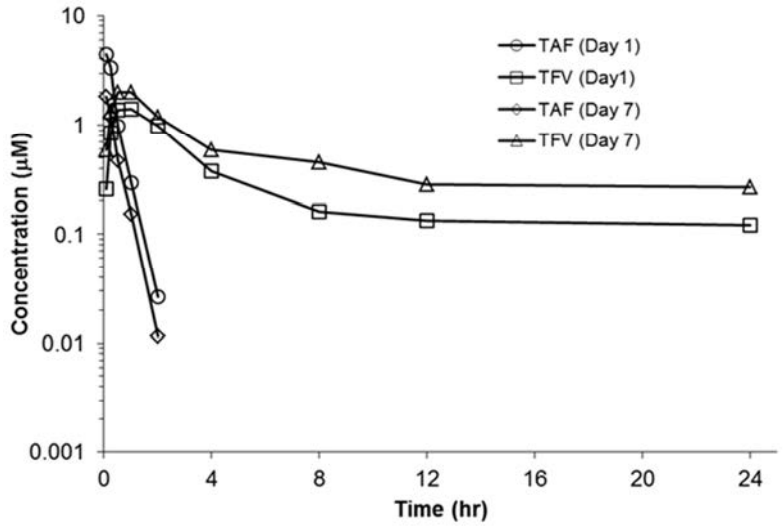
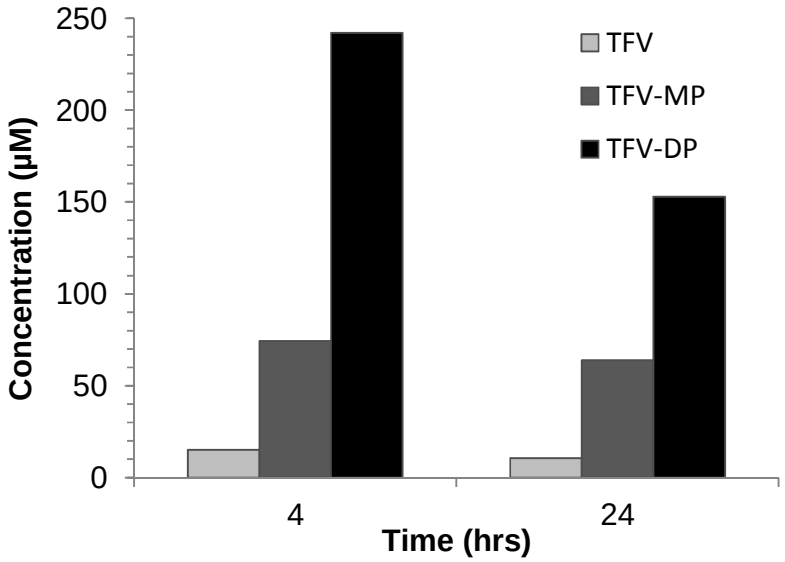


Figure 7. **Liver Concentrations of TFV, TFV-MP, and TFV-DP Following Repeat Dose Oral Administration of TAF on Day 7**



The plasma PK of TAF and TFV as well as TFV levels in PBMCs were determined during the course of a 28-day oral gavage toxicity study in adult male and female beagle dogs following daily administration of either vehicle, 0.1, 0.3, 1.0, 3.0, or 10 mg/kg/day GS-7340-02 (m2.6.5, Section 4.3.2, D990175-PK). Repeat dosing at 10 mg/kg/day resulted in nonlinear PK between Days 1 and 28 with TAF median AUC values of 0.454 and 0.985 µg·h/mL, C_{max} values of 582 and 1280 ng/mL, and $t_{1/2}$ λ_z values of 18 and 23 minutes, respectively. The TFV C_{max} values appeared to be linear with increasing dose as well as repeat dosing. The TFV $t_{1/2}$ was estimated to be 37 hours and substantial accumulation of TFV was observed after repeat dosing. The TFV levels in PBMCs were not linear with increasing dose; however, a linear correlation was observed between TFV levels in PBMCs and corresponding trough plasma concentrations. PBMC concentrations were approximately 100-fold higher than corresponding plasma concentrations.

In a 9-month toxicology study in dogs, GS-7340-02 was administered once daily at doses of 0, 2, 6, and 18 mg/kg/day (m2.6.7, Section 7.3.5, TOX-120-002). The dose of 18 mg/kg/day was decreased to 12 mg/kg/day on Day 2 of Week 7 for males and Day 2 of Week 8 for females due to severe clinical signs and reduced body weight and food consumption. The concentrations of GS-7340 and TFV in plasma samples and total TFV in Week 39/40 PBMC samples were determined. GS-7340 was rapidly absorbed and converted to TFV following oral dose administration, with peak plasma concentrations of GS-7340 and TFV occurring at 0.5 and 1 hour postdose, respectively. GS-7340 was eliminated rapidly from the plasma with a terminal phase half-life of less than 1 hour. The median $t_{1/2}$ of TFV was estimated to be in the range of 25 to 31 hours on Day 1. The plasma PK of GS-7340 and TFV were comparable between male and female dogs after oral administration. Plasma C_{max} and AUC values for TAF increased more than proportionally over the dose range of 2 to 18/12 mg/kg/day. The plasma

TFV C_{max} and AUC increased in an approximately dose proportional manner. There was some accumulation of TFV following repeat dosing (~3-fold). Tenofovir concentrations in PBMCs were measurable at 24-hour postdose for all dose groups. The median terminal phase half-life of total TFV in PBMCs was estimated to be 31 hours (similar to the TFV plasma estimate) from the recovery animals with PBMC concentrations measured up to 72 hours. Dose-normalized PBMC mean AUC values of total TFV increased more than dose proportionally during Week 39/40.

3.3.3.4. Monkey

Following daily oral administration of GS-7340-02 at 0, 3, and 30 mg/kg/day or TFV at 15 mg/kg/day for 28 days, PK profiles of TAF and/or TFV were determined on Day 1, Day 14, and Day 28 (m2.6.5, Section 4.3.3 and m2.6.7, Section 7.3.6, P2000114). Concentrations of TFV in PBMCs were determined on Day 14 and Day 28. No significant differences in PK parameters were found between males and females. The PK parameters for TFV were dose-linear on Day 1 but were greater than dose-linear on Day 28 after oral administration of GS-7340-02. There was no statistically significant accumulation of TFV following repeat dosing of either GS-7340-02 or TFV. The intracellular TFV concentrations in PBMCs were only determined from the 30 mg/kg/day GS-7340-02 dose group where 72.3 and 27.2 µg/mL were detected on Day 14 and Day 28, respectively.

3.4. B/F/TAF

No formal nonclinical studies of the absorption kinetics of the B/F/TAF FDC have been conducted. However, comprehensive clinical studies on the combination product have been performed (m2.7.2, Section 1.2).

4. DISTRIBUTION

4.1. In Vitro Protein Binding

4.1.1. BIC

4.1.1.1. Plasma Protein Binding

The binding of BIC (2 μM) in plasma was determined in vitro with equilibrium dialysis (m2.6.5, Section 6.1.1, AD-141-2287). Bictegravir was highly bound to plasma protein in all species tested (> 98% bound; Table 17).

Table 17. Protein Binding of BIC in Plasma from Different Species

Matrix	Unbound (%)[a]	Bound (%)[a]
Sprague-Dawley Rat Plasma	0.01 ± 0.00	99.99 ± 0.00
Beagle Dog Plasma	1.24 ± 0.06	98.76 ± 0.06
Cynomolgus Monkey Plasma	0.31 ± 0.01	99.69 ± 0.01
Rhesus Monkey Plasma	0.32 ± 0.02	99.68 ± 0.02
Human Plasma	0.25 ± 0.01	99.75 ± 0.01

a Values are the mean ± standard deviation of 3 determinations.
Source: AD-141-2287

4.1.1.2. Relative Protein Binding in Human Plasma and Cell Culture Medium

The relative binding of BIC (2 μM) between human plasma and cell culture media (CCM) was determined by a competitive equilibrium dialysis method (Section 2.1.3.2). At equilibrium the concentration of BIC in human plasma was 43.6-fold higher than in CCM (m2.6.5, Section 6.1.1, AD-141-2287). This ratio was used to obtain the plasma protein binding-adjusted half-maximal effective concentration (EC_{50}) values by multiplying it by the in vitro EC_{50} values measured in CCM (m2.6.3, Section 1.1, PC-141-2032).

4.1.1.3. Protein Binding to Human Microsomal Fraction

The binding of BIC in human hepatic microsomal fraction was determined in vitro using equilibrium dialysis (m2.6.5, Section 6.1.2, AD-141-2311). There was little binding of BIC to the liver microsomes (mean % fraction unbound 86.3%).

4.1.2. FTC

The binding of FTC to mouse, rabbit, monkey, and human plasma was determined over the concentration range 0.020 to 200 μg/mL by equilibrium dialysis at 37ºC (m2.6.5, Section 6.2.1, TBZZ/93/0025). The mean percentage bound for all species studied was ≤ 3.6%, with no indication of concentration dependence.

4.1.3. TAF and TFV

Since TAF is highly unstable in rodent plasma due to high levels of plasma esterases expressed in some rodent species, the extent of TAF binding to plasma could only be determined in dog and human plasma in vitro (m2.6.5, Section 6.3.1, AD-120-2026). Protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. These in vitro values were higher than those observed in multiple human ex vivo studies with the mean percent of unbound TAF ranging from 14% to 23% in all subjects (GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant, the percent of unbound TAF of 20% was used for the assessments for potential drug interactions (Section 7).

The protein binding of TFV has been determined in human plasma and serum using centrifugal ultrafiltration over the range of 0.01 to 25 μg/mL (m2.6.5, Section 6.3.2, P0504-00039.1). The percent of unbound TFV was 99.3 ± 3.3% in human plasma, and 92.8 ± 3.6% in human serum. Tenofovir therefore showed very low protein binding in either human plasma or serum.

4.2. Blood to Plasma Ratio

4.2.1. BIC

The in vitro B/P BIC concentration ratio was close to 0.6, measured as 0.58, 0.60, 0.65, 0.62 and 0.64 for the rat, dog, cynomolgus monkey, rhesus monkey, and human, respectively (m2.6.5, Section 5.1.1, AD-141-2312). The low B/P ratio of BIC suggests minimal binding to erythrocytes.

4.3. Tissue Distribution Studies

4.3.1. BIC

4.3.1.1. Wistar Han and Long Evans Rats

The tissue distribution of BIC following a single oral dose of [^{14}C]BIC at 2 mg/kg to male Wistar Han (non-pigmented) and Long Evans (pigmented) rats was determined by QWBA (m2.6.5, Section 5.1.2, AD-141-2276). A total of 52 tissues were examined and results from representative tissues are shown in Table 18 and Table 19.

The [^{14}C]BIC-derived radioactivity was rapidly (0.25 hours postdose) and widely distributed to most tissues and was similar in both Wistar Han and Long Evans rat. The autoradiograph of Long Evans rats 1 hour following a single oral dose is shown in Figure 8. Concentrations in tissues were lower than in blood and decreased throughout the course of the study (168 hours). Low levels of radioactivity were detected in brain (< 4% relative to blood), suggesting that [^{14}C]BIC-derived radioactivity poorly crossed the blood brain barrier. By 168 hours, quantifiable radioactivity was observed in tissues, but concentrations were declining, suggesting reversible binding. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]BIC-related radioactivity was not selectively associated with melanin-containing tissues.

Figure 8. Annotated Whole-Body Autoradiograph at 1 Hour After a Single Oral Administration of [^{14}C]BIC to a Male Long Evans Rat (2 mg/kg, 100 µCi/kg) (Animal B39349)

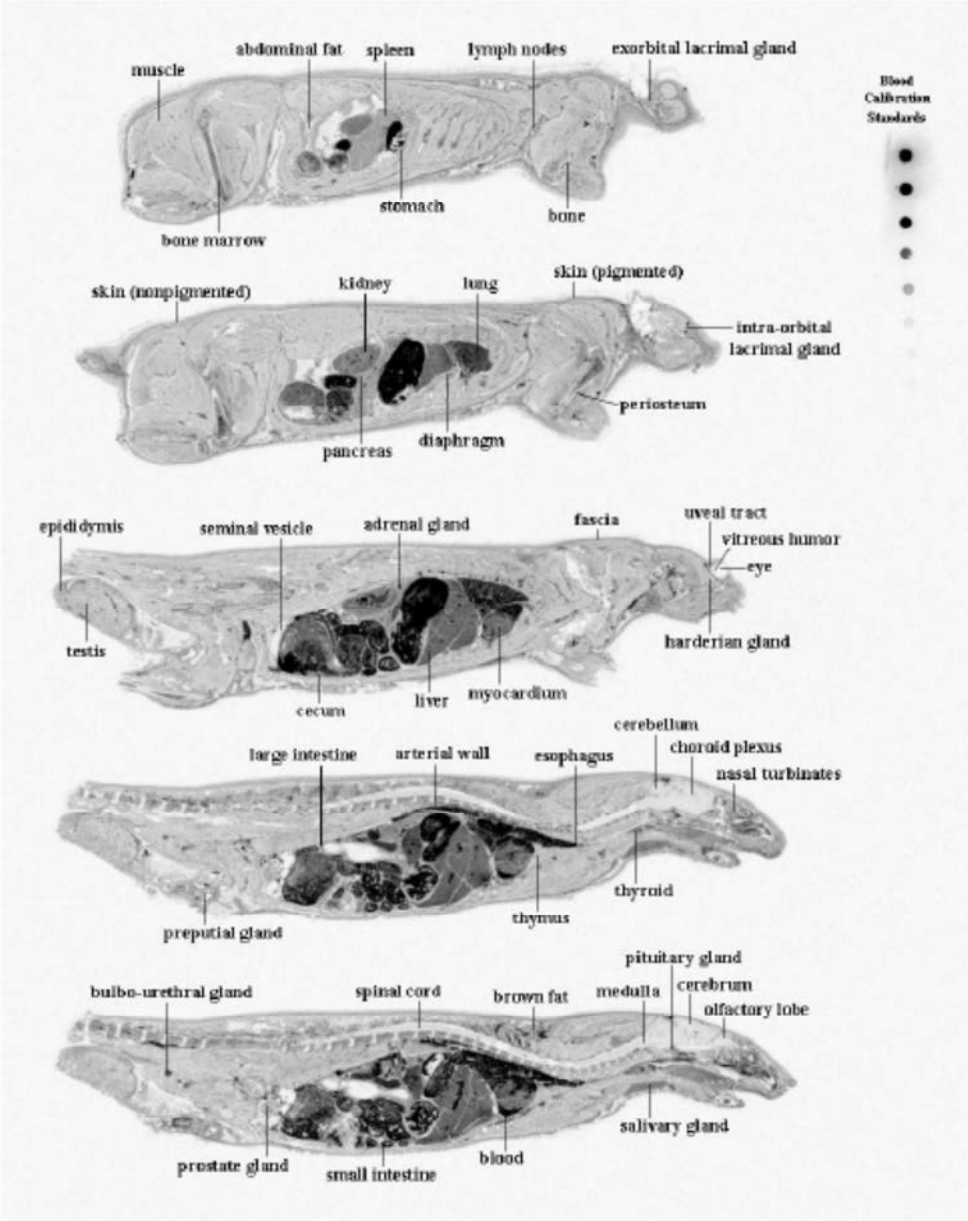


Table 18. Concentrations of Radioactivity in Blood and Selected Tissues Determined by QWBA After a Single Oral Administration of [^{14}C]BIC to Male Wistar Han Rats at 2 mg/kg (100 µCi/kg)

	ng [^{14}C]BIC Equivalents/g Tissue					
Tissue/Matrix	**1 h**	**4 h**	**12 h**	**48 h**	**96 h**	**168 h**
Adrenal gland	4170	2890[a]	1250	719	96.7[a]	246
Bile	8310	6350	4480	1400	ND	ND
Blood	18700	10000	7530	3640	473	709
Bone	282	190	87.2	70.0	BLQ	BLQ
Bone marrow	3820	2250	1520	576	66.4	103
Brain medulla	150	121	86.9	37.5	ND	ND
Eye uveal tract	914	1570	1130	655	128	170
Eye	341	614	288	229	32.7	69.5
Fat (brown)	2810	1990	923	483	135	131
Kidney	3210	2940	1850	872	109	213
Large intestine	668	2210	2780	716	145	200
Liver	3290	2860	1110	869	498	270
Pancreas	1540	1600	969	390	59.4	107
Skin (nonpigmented)	243	1400	1650	958	195	266
Small intestine	8050	2800	1120	436	89.8	98.4
Spinal cord	249	196	95.7	26.8	ND	ND
Stomach	1030	1280	2360	888	96.2	136
Testis	943	2600	1300	699	74.4	136
Urinary bladder	905	2340	3720	2520	305	ND
Urine	238	281	446	236	95.5	ND

BIC = bictegravir (GS-9883); BLQ = below the limit of quantitation (<18.7 ng equivalents ^{14}C-GS-9883/g); h = hours;
ND = not detectable (sample shape not discernible from background or surrounding tissue); QWBA = quantitative whole body autoradiography
a Tissue appeared to be fat soaked.
Source: AD-141-2276

Table 19. Concentrations of Radioactivity in Blood and Selected Tissues Determined by QWBA After a Single Oral Administration of [^{14}C]BIC to Male Long Evans Rats at 2 mg/kg (100 µCi/kg)

	ng [^{14}C]BIC Equivalents /g Tissue					
Tissue/Matrix	**1 h**	**4 h**	**12 h**	**48 h**	**96 h**	**168 h**
Adrenal gland	2820	2750	979	978	349[a]	310[a]
Bile	7430	5120	2670	4190	ND	ND
Blood	11500	12500	7790	4430	1860	1320
Bone	169	211	108	76.7	22.8	BLQ
Bone marrow	2350	2700	1320	683	252	161
Brain medulla	134	143	83.9	60.9	20.2	BLQ
Eye uveal tract	2120	3830	2780	1960	687	383
Eye	224	552	372	271	99.1	60.5
Fat (brown)	1490	2320	879	798	240	204
Kidney	2950	3460	1570	1130	551	313
Large intestine	1580	2360	2650	1280	560	269
Liver	4110	3690	1100	567	491	314
Pancreas	1400	1630	895	643	251	157
Skin (nonpigmented)	458	670	1210	1340	655	350
Skin (pigmented)	468	948	1540	1300	747	385
Small intestine	5590	2610	1280	527	366	212
Spinal cord	249	129	73.5	49.5	25.1	BLQ
Stomach	1560	1500	1370	796	255	161
Testis	669	2030	1110	1020	319	203
Urinary bladder	ND	1270	1240	2460	1220	582
Urine	ND	155	151	104	82.4	BLQ

BIC = bictegravir (GS-9883); BLQ = below the limit of quantitation (<18.7 ng equivalents ^{14}C-GS-9883/g); h = hours;
ND = not detectable (sample shape not discernible from background or surrounding tissue); QWBA = quantitative whole body autoradiography
a Tissue appeared to be fat soaked.
Source: AD-141-2276

4.3.2. FTC

4.3.2.1. Rat

To examine brain penetration of FTC, male Sprague-Dawley rats were administered FTC intraperitoneally at 10 mg/kg, and brain and plasma concentrations were determined. Brain/plasma ratios were low (3.4%–6.9%) and were unaffected by pretreatment with probenecid (60 mg/kg 15 minutes prior to treatment with FTC) {Frick 1993}.

Male Sprague-Dawley nonpigmented rats and Long Evans pigmented rats received a single 200 mg/kg oral dose containing approximately 135 μCi/kg of [^{14}C]FTC via gavage (m2.6.5, Section 5.2.1, TOX092). The distribution of radioactivity in the nonpigmented tissues of Long Evans pigmented rats was similar to that of Sprague-Dawley rats (56 tissues assessed). For both groups, the absorption of [^{14}C]FTC following oral administration was rapid, and the radioactivity was widely distributed among all of the examined tissues. The clearance of radioactivity from the plasma and tissues was also rapid with a $t_{1/2}$ of approximately 1 to 6 hours for blood and tissues, and 2.7–3.4 hours for plasma. In agreement with the previous study with unlabeled FTC, tissue:plasma ratios for radioactivity in CNS tissues were all < 0.1.

The PK parameters for [^{14}C]FTC derived radioactivity in eyes and skin were not markedly different for nonpigmented and pigmented rats, indicating that [^{14}C]FTC-associated radioactivity does not bind appreciably to melanin.

4.3.2.2. Monkey

To assess brain penetration of FTC, cynomolgus monkeys were dosed orally with 40, 200, and 1000 mg/kg of FTC and concentrations were determined in plasma and CSF. Concentrations of FTC in the CSF were dose- and concentration-independent and were 4% ± 0.7% of the corresponding levels in plasma {Frick 1994}.

Male cynomolgus monkey received an oral dose of 200 mg/kg FTC containing 42.9 μCi/kg [^{14}C]FTC. The tissue distribution of radioactivity after oral administration of [^{14}C]FTC in the cynomolgus monkey is described in detail in Section 5.3.2 (m2.6.5, Section 8.2.2, TOX063). Radioactivity was widely distributed to all tissues by 1 hour postdose. Plasma concentrations of radioactivity declined in parallel of those of parent FTC. Concentrations of radioactivity in tissues were similar to those in plasma except for the gastrointestinal (GI). The mean whole blood to plasma ratio was 0.87.

4.3.3. TAF

4.3.3.1. Mouse

After oral dosing of 100 mg/kg [^{14}C]TAF to male CD-1 mice, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues by the first collection time point (0.5 hours postdose) (m2.6.5, Section 5.3.1, AD-120-2011). Most tissues reached maximum concentration by 1 hour postdose. The tissues showing the highest maximum concentrations of radioactivity, excluding GI tract, included liver, gall bladder, urinary bladder, diaphragm, kidney cortex, kidneys, and kidney medulla. The tissues with the lowest C_{max} values were testis, brain cerebrum, fat (abdominal), spinal cord, and brain medulla.

In male C57 Black mice, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues by the first collection time point (0.5 hours postdose), similar to CD-1 mice. Most tissues reached maximum concentration by 0.5 hours postdose. The tissues showing the highest maximum concentrations of radioactivity, excluding the GI tract, included liver, gall bladder, urinary bladder, kidney cortex, kidneys, kidney medulla, and diaphragm. The tissues with the lowest C_{max} values were testis, spinal cord, brain cerebrum, brain medulla, and brain cerebellum.

Low levels of radioactivity were detected in brain in mice suggesting [^{14}C]TAF-derived radioactivity poorly crossed the blood:brain barrier. Low levels of radioactivity were also measured in testis in mice, suggesting [^{14}C]TAF-derived radioactivity poorly crossed the blood:testis barrier.

More persistent exposures in eye lens, eye uveal tract, and eyes were observed in CD57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that ^{14}C-TAF-related radioactivity was not selectively associated with melanin-containing tissues. Comparative quantification data for selected time points are provided in Table 20.

Table 20. Comparative Tissue Concentrations of Radioactivity in Male CD-1 and C57 Black Mice After Oral Administration of [^{14}C]TAF (n = 1 per time point)

Organ	Radioactivity (µg equivalents [^{14}C]TAF/g tissue)									
Time point	0. 5 h		1 h		3 h		12 h		24 h	
Rat Strain	CD-1	C57 Black	CD-1	C57 Black	CD-1	C57 Black	CD-1	C57 Black	CD-1	C57 Black
Adrenal gland(s)	9.92	34[a]	27.5	26.4	9.45	20.4	3.01	7.75[a]	3.6	10.4[a]
Bile	60.3	198	127	136	115	150	65.9	40.3	12.9	26.6
Blood	14.6	16.7	14.5	9.27	5.28	7.91	4.38	4.17	6.1	3.78
Bone	1.3	11.2	2.42	4.49	1.23	4.9	1.01	2.43	0.525	1.77
Bone marrow	4.73	24.3	6.61	19	2.03	16.1	2.04	6.44	2.12	6.47
Brain cerebellum	0.569	0.611	1.94	0.59	0.395	BLQ	BLQ	ND	BLQ	ND
Brain cerebrum	0.768	1.24	1.43	0.661	BLQ	BLQ	0.379	ND	BLQ	ND
Brain medulla	0.42	0.886	0.833	BLQ	BLQ	BLQ	BLQ	ND	BLQ	ND
Brain olfactory lobe	1.12	3.79	2.89	1.73	0.464	1.64	0.461	0.688	BLQ	0.707
Cecum	3.44	25.4	6.96	95.4	90.5	ND[b]	19.1	50.5	8.31	10.5
Diaphragm	40.6	60.3	167	103	46.6	114	26.6	27.5	28.3	25.9
Epididymis	3.6	10.8[a]	22.3	4.19	1.31	4.99	0.54	2.12	0.864	2.15[a]
Esophagus	38.4	76.1	90.8	81.3	57.3	48.9	20.1	27.9	8.66	8.54
Exorbital lacrimal gland	3.6	18.1	5.46	12	1.4	11.8	1.25	4.91	1.29	3.53
Eye lens	0.967	4.31	2.98	1.87	0.508	1.11	BLQ	0.527	BLQ	BLQ
Eye uveal tract	4.18	13.4	3.69	12.2	0.754	11.6	0.947	4.74	0.779	6.14
Eye(s)	1.88	5	2.66	2.92	0.539	2.44	0.428	1.04	0.363	1.06
Fat (abdominal)	1.25	5.46	1.17	6.82	0.968	2.14	0.512	4.18	0.787	1.7
Fat (brown)	3.07	19.2	5.59	18.7	2.53	14.5	2.06	10.8	3.03	8.66
Gall bladder	335	163	216	379	108	275	68.1	94	37.1	39.8
Harderian gland	4.24	18.5	6.5	14.6	1.53	13.6	2.17	6.2	1.39	4.58
Intra-orbital lacrimal gland	7.63	NR	3.58	NR	1.9	NR	2.28	NR	1.5	3.76
Kidney cortex	92.3	137	89	125	74	104	30	58	23.1	34.5

Organ	Radioactivity (µg equivalents [^{14}C]TAF/g tissue)									
Time point	**0. 5 h**		**1 h**		**3 h**		**12 h**		**24 h**	
Rat Strain	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**
Kidney medulla	65	125	70	94.7	54.8	80	18.1	40.2	12.8	19.4
Kidney	84.8	132	86.1	107	68.9	89.6	25.9	47.5	19.9	29.5
Large intestine	6.68	25.7	8.09	17.3	7.73	32.3	22.5	74.2	6.77	35
Liver	282	488	447	490	290	385	197	282	164	118
Lung	13.2	32.3	23.9	32.5	4	26.5	10	18.1	11.5	13.5
Lymph node(s)	5.19	17.9	7.75	14.6	1.68	12.6	1.76	8.02	2.28	10.9
Muscle	1.68	8.78	2.1	4.14	0.466	3.19	0.768	2.01	0.579	3.9
Myocardium	7.57	23.2	11.7	13.5	2.38	13.6	4.6	8.47	4.62	9.51
Nasal turbinates	2.72	10.6	6.44	2.98	1.29	3.55	0.983	2.39	1.19	1.92
Pancreas	6.13	34.9	14.5	26.9	2.95	31.5	3.54	15.4	2.88	13.9
Pituitary gland	2.91	17.4	7.44	8.44	1.28	6.59	2.51	0.85	1.3	ND
Preputial gland	1.9	11.5	2.58	6.39	0.635	6.13	0.708	2.24	0.503	1.97
Prostate gland	5.5	ND[c]	2.88	8.63	2.15	4.84	3.06	8.62	2.59	ND
Salivary gland	5.39	36.2	11.5	24.2	1.88	27.2	2.42	9.91	2.16	12.2
Seminal vesicle	1.79	5.54	3.41	3.53	0.957	2.47	0.852	2.15	0.62	0.825
Skin (nonpigmented)	8.52	NA	5.58	NA	1.51	NA	0.674	NA	0.715	NA
Skin (pigmented)	NA	11.6	NA	5.93	NA	3.35	NA	1.18	NA	1.34
Small intestine	8.77	26.9	74.5	56.1	88.1	28	14.1	14.1	4.04	11.3
Spinal cord	0.757	1.95	1.21	0.742	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Spleen	6.1	35.3	12.9	29.6	3.85	29.1	5.46	16.6	6.27	12.2
Stomach	77.6	60.5	39.3	70.6	26.6	29.2	5.46	7.9	5.94	6.73
Stomach mucosa	96.9	ND	46.9	ND	19.8	ND	5.28	ND	3.66	ND
Stomach wall	48.6	ND	24.5	ND	26.6	ND	7.69	ND	22.7	ND
Testis	1.16	2.64	1.49	1.76	0.774	1.07	BLQ	0.512	0.319	0.752
Thymus	2.51	12.4	6.14	8.19	0.914	7.19	0.903	3.44	1.19	3.41
Thyroid	7.12	40.7	12.1	32.7	2.83	29	1.2	NR	4.6	10.9
Urinary bladder	ND[c]	ND[c]	174	138	85.6	49.1	5.8	12	10.3	6.29
Urine	1790	1170	413	626	200	128	36.8	135	24.7	18.4

BLQ = below the limit of quantitation [< 311 (CD-1) or < 490 (C57 Black) µg equivalents [^{14}C]TAF/g]; h = hours;
NA = not applicable; ND = not detectable (sample shape not discernable from background or surrounding tissue);
NR = not represented (tissue not present in section).

a Tissue appeared to be fat-soaked.
b Not detectable due to flare from cecum contents.
c Not detectable due to flare from urine.

4.3.3.2. Rat

After oral dosing of 5 mg/kg [^{14}C]TAF to male Sprague-Dawley or Long Evans rats, [^{14}C]TAF-derived radioactivity was widely distributed to most tissues by the first collection time point (0.25 hours postdose) (m2.6.5, Section 5.3.2, AD-120-2020). Representative autoradiographic images from animals terminated at 0.25 hours postdose are provided in Figure 9 (nonpigmented) and Figure 10 (pigmented). Comparative quantification data for selected time points are provided in Table 21. A full listing of tissue concentrations of radioactivity is provided in tabular form in m2.6.5 (Section 5.3.2 for albino animals and pigmented animals). In both Sprague-Dawley and Long Evans rats, most tissues reached maximum concentrations by the first collection time point. The tissues showing the highest maximum concentrations of radioactivity included kidney cortex, kidney(s), kidney medulla, and liver.

The tissues with the lowest C_{max} values were brain olfactory lobe, seminal vesicle(s), eye vitreous humor, thymus, eyes, testis(es), and harderian gland for Sprague-Dawley rats and bone, brain olfactory lobe, seminal vesicle(s), fat (abdominal), muscle, eye vitreous humor, and eye(s) for Long Evans rats. Transient exposure to low levels of [^{14}C]TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin.

Table 21. Comparative Tissue Concentrations of Radioactivity in Male Sprague-Dawley and Long Evans Rats After Oral Administration of [^{14}C]TAF (n = 1 per time point)

Organ	Radioactivity (ng equivalents [^{14}C]TAF/g tissue)									
Time point	0.25 h		1 h		4 h		12 h		24 h	
Rat Strain	SD	LE	SD	LE	SD	LE	SD	LE	SD	LE
Adrenal gland(s)	181[a]	353[a]	129[a]	115	BLQ[a]	48.5[a]	ND	ND	ND	ND
Arterial wall	817	1350	299	270	118	90.4	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1070	1260	334	221	138	116	83.1	117	ND	ND
Bone	BLQ	50.4	ND	BLQ	ND	ND	ND	ND	ND	ND
Bone marrow	233	311	125	84.9	72.6	BLQ	BLQ	BLQ	ND	ND
Brain cerebellum	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	45.7	57.2	BLQ	51.0	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	396	831	177	209	236	ND	ND	ND	ND	ND
Cecum	218	603	132	118	541	889	NR	362	323	494
Diaphragm	210	353	145	124	52.9	55.8	ND	ND	ND	ND
Epididymis	249[a]	516	101[a]	79.3	BLQ	BLQ	ND	ND	ND	ND
Esophagus	341	923	222	218	187	186	58.9	65.8	ND	ND
Exorbital lacrimal gland	234	353	101	56.1	51.2	BLQ	ND	ND	ND	ND
Eye lens	BLQ	BLQ	ND	BLQ	ND	ND	ND	ND	ND	ND

Organ	Radioactivity (ng equivalents [^{14}C]TAF/g tissue)									
Time point	**0.25 h**		**1 h**		**4 h**		**12 h**		**24 h**	
Rat Strain	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**
Eye uveal tract	409	555	187	89.5	78.4	59.4	ND	ND	ND	ND
Eye vitreous humor	84.9	139	89.3	BLQ	BLQ	BLQ	ND	ND	ND	ND
Eye	86.1	150	92.7	BLQ	BLQ	BLQ	ND	ND	ND	ND
Fat (abdominal)	BLQ	70.0	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Fat (brown)	200	232	97.1	BLQ	48.2	ND	55.7	ND	ND	ND
Harderian gland	98.6	209	52.4	BLQ	BLQ	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	250	402	118	53.4	BLQ	49.1	ND	ND	ND	ND
Kidney cortex	10700	8000	12400	8890	11800	6980	8300	5150	2010	2440
Kidney medulla	8240	6900	5040	3670	2800	655	1010	757	317	367
Kidney	9520	7750	8710	7570	8250	5160	4590	3000	1380	1310
Large intestine	364	548	140	91.5	55.8	474	ND	133	ND	132
Liver	6730	10300	6730	7800	4010	7710	3570	5610	1090	1380
Lung	592	854	211	145	81.1	67.2	ND	66.3	ND	BLQ
Lymph node	318[a]	422[a]	ND	94.9[a]	ND	ND	ND	ND	ND	ND
Muscle	101	115	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Myocardium	361	512	136	46.6	58.6	BLQ	ND	BLQ	ND	ND
Nasal turbinates	123	201	83.8	71.9	52.8	BLQ	ND	ND	ND	ND
Pancreas	215	274	120	82.1	68.9	53.5	52.0	ND	ND	ND
Pituitary gland	335	434	116	53.1	50.5	ND	76.2	ND	ND	ND
Preputial gland	140[a]	247	60.1[a]	67.9	BLQ	ND	ND	ND	ND	ND
Prostate gland	134	247	88.6	BLQ	117	49.9	BLQ	ND	ND	ND
Salivary gland	292	368	119	85.3	BLQ	BLQ	ND	BLQ	ND	ND
Seminal vesicle	62.7	67.7	ND	BLQ	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	393	526	123	71.8	BLQ	BLQ	ND	ND	ND	ND
Skin (pigmented)	NA	623	NA	78.9	NA	BLQ	NA	ND	NA	ND
Small intestine	479	530	500	376	364	122	86.5	220	171	98.6
Spinal cord	BLQ	BLQ	ND	BLQ	ND	ND	ND	ND	ND	ND
Spleen	147	231	105	83.1	59.0	58.3	62.8	60.7	BLQ	BLQ
Stomach	475	682	126	113	66.6	71.4	ND	159	ND	BLQ
Testis	98.2	157	50.9	BLQ	BLQ	BLQ	ND	BLQ	ND	ND
Thymus	91.2	181	68.1	BLQ	BLQ	BLQ	ND	ND	ND	ND
Thyroid	324	412	147	59.1	63.6	ND	ND	ND	ND	ND
Tooth pulp	646	793	228	194	84.4	78.7	ND	57.0	ND	NR
Urinary bladder	925	352[a]	205	155	ND[b]	125	1050	45.9	BLQ	48.3
Urine	112000	50200	34400	61000	51100	2280	14500	1140	988	1500

BLQ = below the limit of quantitation (<45.6 ng equivalents [^{14}C]TAF/g); h = hours; NA = not applicable; ND = not detectable (sample shape not discernable from background or surrounding tissue).

a Tissue appeared to be fat-soaked.

b Not detectable due to flare from urine.

Figure 9. Annotated Whole-Body Autoradiograph for a Male Sprague-Dawley Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg

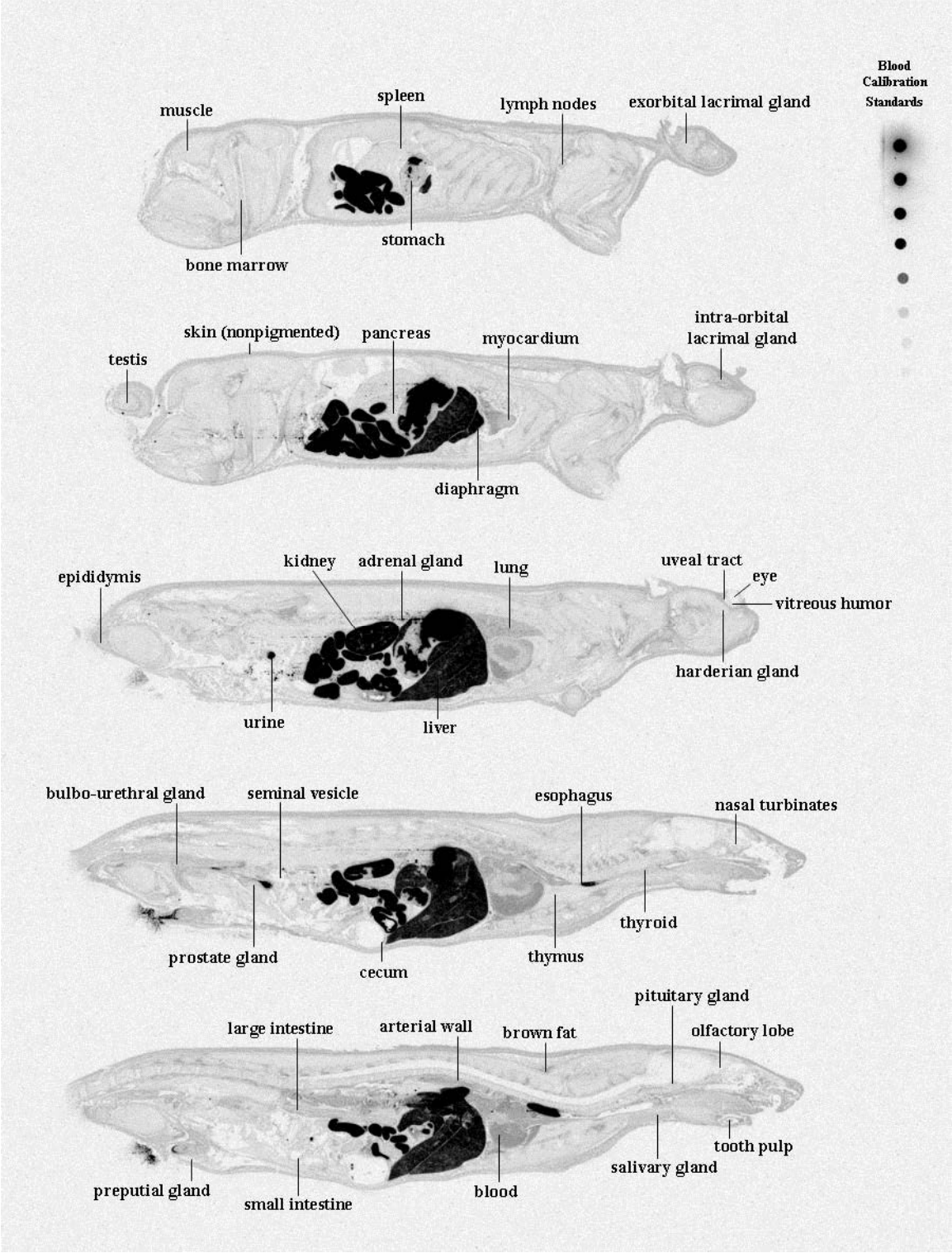
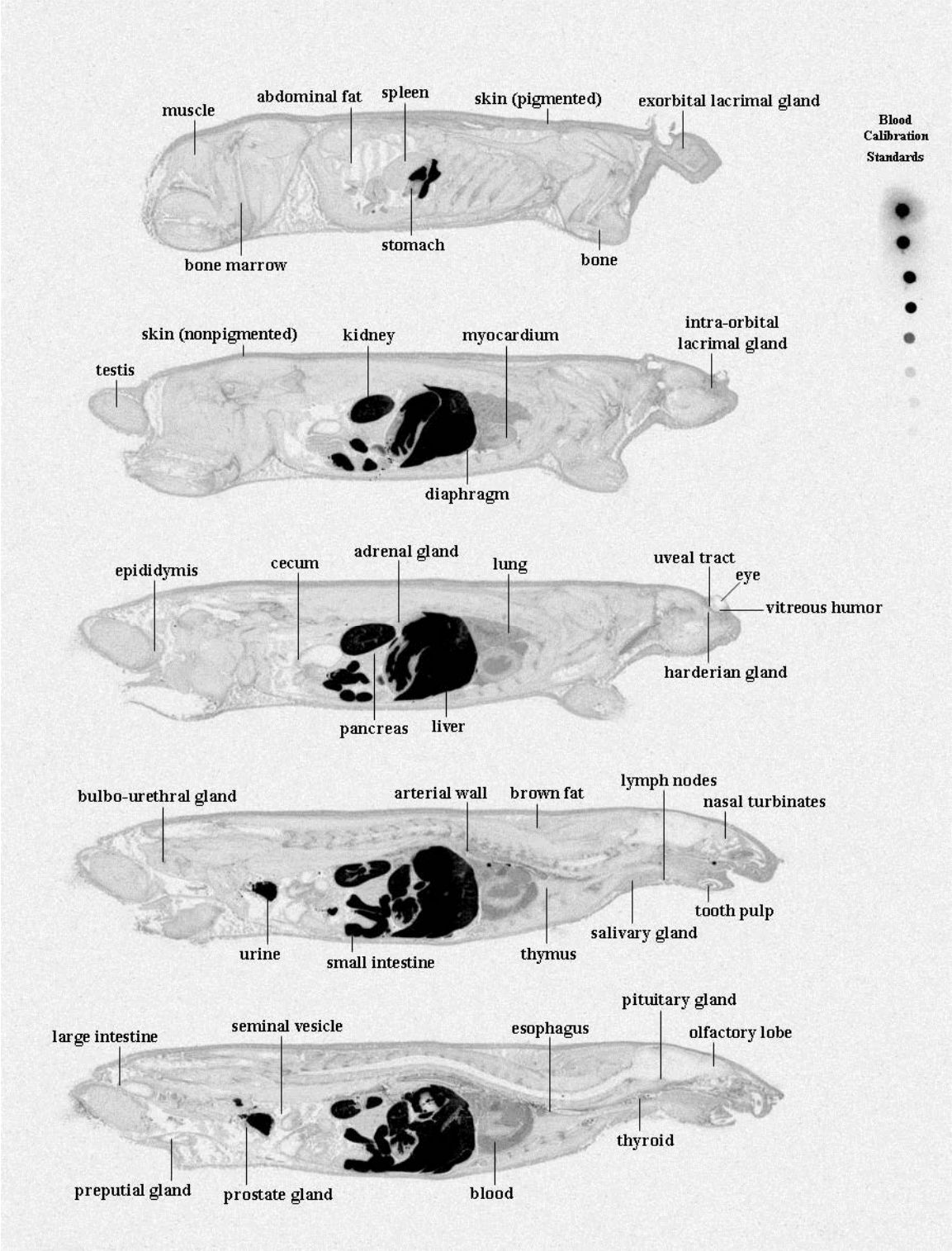


Figure 10. Annotated Whole-body Autoradiograph for a Male Long Evans Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg



4.3.3.3. Dog

The absorption and distribution of [^{14}C]TAF were determined following multiple 15-mg/kg oral doses of GS-7340 and a single oral dose of [^{14}C]TAF to male dogs at 15-mg/kg or at 18.1 mg/kg (m2.6.5, Section 5.3.3, AD-120-2009 and m2.6.5, Section 5.3.4, D990173-BP). Radioactivity was widely distributed but not detected in brain, eye, and CSF. High concentrations were observed in kidney, liver, GI tract, spleen, lymph nodes and PBMCs. Following multiple or single oral administrations of TAF or [^{14}C]TAF to male dogs, the highest concentrations of radioactivity were observed in the liver and kidney through 24 hours postdose. Concentrations of radioactivity in tissues after multiple doses of unlabeled GS-7340 followed by a single dose of [^{14}C]TAF were higher compared to tissues from animals dosed only with a single dose of [^{14}C]TAF.

4.4. Studies in Pregnant or Nursing Animals

4.4.1. BIC

Toxicokinetic parameters for BIC were determined in pregnant rats (m2.6.7, Section 11.1, TX-141-2034 and m2.6.7, Section 14.1, TX-141-2045) and rabbits (m2.6.7, Section 11.1, TX-141-2038). The plasma exposures were comparable between the pregnant and the non-pregnant animals in both species.

The plasma exposure of BIC in nursing pups was determined in a prenatal and postnatal development study in rats (m2.6.7, Section 14.1, TX-141-2045). Bictegravir was detected in the plasma of neonates on lactation day 10. Bictegravir exposure in maternal rats was roughly similar to pups at the 2 mg/kg/day dose level, slightly higher (approximately 1.5-fold) in maternal rats than in pups at the 10 mg/kg/day dose level, and greater than 2-fold higher (approximately 2.8-fold) in maternal rats than in pups at the 300 mg/kg/day dose level. These data suggested that BIC present in maternal rat systemic circulation was distributed to milk and transferred to nursing pups.

4.4.2. FTC

4.4.2.1. Pregnant Mice

Following oral administration of FTC to pregnant mouse dams, the exposure of murine fetuses to FTC was examined (m2.6.5, Section 7.2.1, TOX103 and Addendum report). Emtricitabine suspended in the vehicle, 0.5% methylcellulose (aqueous), was administered orally by gavage to Crl:CDR®-1(TCR) BR mice twice daily (approximately 6 hours between doses) from gestation Days 6 to 14 at a dose of 1000 mg/kg/day administered at a dose volume of 5 mL/kg/dose. On gestation Day 15, following administration of 500 mg/kg of FTC twice daily for approximately 10 days, pregnant mice and their viable fetuses had measurable concentrations of FTC 1 hour following administration of the first 500 mg/kg dose. The mean plasma concentration in the pregnant mice was 137.1 ± 28.0 µg/mL. The mean concentration of FTC in pooled fetal homogenate was 55.7 ± 10.4 µg/g. The mean fetal/maternal concentration ratio was 0.41 ± 0.04.

4.4.2.2. Pregnant Rabbit

In an oral GLP embryo-fetal toxicity study, pregnant NZW rabbits (20/dose) were given FTC at 0, 100, 300, and 1,000 mg/kg/day in 0.5% aqueous methylcellulose as equal divided doses 6 hours apart on gestation Days 7 to 19. The does were necropsied on gestation Day 19 (m2.6.5, Section 7.2.2, TOX038 and Addendum report). Additional pregnant rabbits were dosed in parallel to provide plasma for systemic exposure assessment on gestation Day 19. They were killed at 1 hour postdose on Day 20 to provide maternal/fetal blood samples to confirm fetal exposures.

Emtricitabine was rapidly absorbed in dams with C_{max} occurring generally within 1 hour postdose. Systemic exposure to FTC (AUC and C_{max}) increased linearly with dose from 100 to 1000 mg/kg/day in both dams and fetuses. On gestation Day 19, AUC_{0-24} in dams was 87, 315, and 1258 μg·h/mL at 100, 300, and 1000 mg/kg/day, respectively. Plasma elimination $t_{1/2}$ was 3 to 4 hours at all dose levels. Fetal/maternal exposure ratios, as determined by analysis of umbilical cord blood, were around 0.4 to 0.5 at 1 hour after dosing (at T_{max}) for all dose levels. Emtricitabine was therefore readily transferred across the placenta (TOX038 and Addendum report).

4.4.3. TAF and TFV

4.4.3.1. TAF: Pregnant Rats

Tenofovir concentrations were determined in plasma from pregnant female rats dosed with GS-7340-02 by oral gavage for at least 12 days (gestation days [GDs] 6 to 17) at 5, 100, and 200 mg/kg as an oral range-finding study (m2.6.7, Section 11.3, TX-120-2001), or at 25, 100, and 250 mg/kg as an embryo-fetal development study (m2.6.7, Section 13.5, TX-120-2002). Blood samples were collected on GDs 6 and 17. Following oral gavage of GS-7340, concentrations of TFV readily appeared in plasma. Exposure to TFV increased with the increase in dose. The increases in C_{max} and AUC_{0-t} were generally greater than dose proportional between the 5 to 200 mg/kg/day dose levels in the range-finding study. In the embryo-fetal development study, C_{max} and AUC_{0-t} were inconsistently proportional between the 25 to 250 mg/kg/day dose levels. While accumulation of TFV was observed after multiple dosing of GS-7340-02 in pregnant rats in the range-finding study, no accumulation of TAF and TFV was observed in the embryo-fetal development study.

4.4.3.2. TAF: Pregnant Rabbits

The TK of TAF and TFV were determined in plasma from pregnant female rabbits following administration of GS-7340-02 via oral gavage once daily on GDs 7 through 20. GS-7340-02 was administered at dose levels of 5, 25, 50, and 100 mg/kg/day as an oral range-finding study (m2.6.7, Section 11.3, TX-120-2004) or at 10, 30, and 100 mg/kg/day as an embryo-fetal development study (m2.6.7, Section 13.6, TX-120-2005). Blood samples were collected from all animals on GDs 7 and 20 predose and approximately 30 minutes and 2, 4, 8, and 24 hours postdose. Tenofovir alafenamide increased with the increase in GS-7340-02 dose level among all groups. The increases in C_{max} and AUC_{0-t} were greater than dose proportional in all dose levels in both studies. Exposure to TFV increased with the increase in GS-7340-02 dose level and the

increases in C_{max} and AUC_{0-t} were approximately proportional in all dose levels. No unexpected accumulation of TFV was observed after repeat dosing of GS-7340-02 in rabbits. GS-7340 was extensively converted to TFV in rabbits following oral administration of GS-7340-02.

4.4.3.3. TFV: Pregnant Monkey

Placental transfer of TFV following subcutaneous administration to a pregnant rhesus monkey was determined (m2.6.5, Section 7.3.2.1, 96-DDM-1278-005). One rhesus monkey received daily subcutaneous injection of 30 mg/kg/day TFV, beginning at Day 111 of gestation. Maternal and fetal blood samples were drawn at Days 115, 127, 134, 140, and 151 of gestation. Placental transfer of TFV appeared to be significant with a fetal/maternal serum concentration ratio of 0.17 ± 0.07 (mean ± SD) at approximately 30 minutes postdose.

4.4.3.4. TFV: Lactating Monkeys

The PK parameters of TFV were investigated in 2 healthy adult lactating rhesus monkeys which were administered a single 30 mg/kg subcutaneous dose of TFV (m2.6.5, Section 7.3.2.2, P2000116). Following dosing, serum TFV C_{max} values of 18.3 and 30.2 µg /mL were observed in the 2 monkeys. Absorption was rapid, with T_{max} occurring at 0.5 hour. As observed in other species, elimination was biphasic, with apparent half-lives of 3.97 and 2.85 hours for the 2 animals. While this appears shorter than the approximately 9-hour terminal half-life observed in male and nonlactating female monkeys (m2.6.5, Section 3.3.10, P2000031) it may have resulted from the more limited period of sampling (24 versus [vs] 48 hours) in the present experiment.

Serum $AUC_{0-\infty}$ values in this study were 68.9 and 56.2 µg·h/mL for the 2 animals. In comparison to AUC values obtained following 30 mg/kg IV doses of TFV (P2000031), these data suggest essentially complete absorption of TFV after subcutaneous administration.

4.4.3.5. TFV: Immature monkeys

The PK parameters of TFV have been determined in infant rhesus monkeys following subcutaneous administration (m2.6.5, Section 7.3.2.1, 96-DDM-1278-005). Tenofovir was formulated as an aqueous solution and was evaluated in monkeys in 4 age groups (newborn, 1, 3, and 12 months old; n = 2 per group). Tenofovir was administered as a 30-mg/kg injection into the dorsal subcutis region. Plasma samples were obtained over the course of 24 hours and concentrations of TFV were determined by HPLC following fluorescence derivatization. The mean TFV C_{max} values in newborn, 1, 3, and 12-month-old monkeys were 51.8, 30.7, 34.6, and 18.8 µg/mL, respectively; with a T_{max} of 0.5 hour for all age groups. The corresponding plasma clearance (CL/F) of TFV was 0.18, 0.54, 0.41, and 1.02 L/h/kg, respectively, showing an increase in the clearance from birth through 1 year. These results suggest that, at an equivalent dose, younger monkeys received greater TFV exposure. The clearance of TFV was dependent on both the weight and the age of the infant monkeys. It is likely that newborn monkeys lack the anion transport system responsible for tubular secretion of TFV.

4.5. B/F/TAF

No nonclinical distribution studies have been performed with the combination of BIC, FTC, and TAF. Coadministration of BIC, FTC, and TAF is not anticipated to alter the distribution profile of the drugs when administered as individual agents.

5. METABOLISM

5.1. Metabolism In Vitro

5.1.1. BIC

The rate of metabolism of [^{3}H]BIC (1 μM), assessed by loss of parent drug, was determined in incubations of pooled hepatic microsomal fractions obtained from human and nonclinical species in the presence of NADPH and UDPGA (m2.6.5, Section 9.1.1, AD-141-2289) and the data are summarized in Table 22. Bictegravir exhibited high metabolic stability (< 30% predicted hepatic extraction). The predicted human hepatic blood clearance, without consideration of plasma binding, was low.

Table 22. In Vitro Rates of Metabolism of BIC by Hepatic Microsomal Fractions

Species	In Vitro $t_{1/2}$ (min)	Predicted Hepatic CL (L/h/kg)	Predicted Hepatic Extraction (%)
Sprague-Dawley Rat	49	1.21	29
Beagle Dog	108	0.29	16
Cynomolgus Monkey	63	0.43	27
Rhesus Monkey	76	0.41	18
Human	194	0.17	13

Source: AD-141-2289

Further studies were performed using cryopreserved hepatocytes incubated for 4 hours with radiolabeled BIC to identify metabolites, determine their abundance and compare nonclinical species with human. The percentage of parent drug and identified metabolites following incubation with [^{14}C]BIC (20 μM) in cryopreserved hepatocytes are summarized in Table 23 and their proposed identities are shown in Figure 12 (m2.6.5, Section 9.1.4, AD-141-2288). Analytes were assigned metabolite numbers (M305, M465, etc.) based on their molecular weight. Metabolic pathways included hydroxylation (3 variants), N-dealkylation, and direct glucuronidation. All human metabolites were also observed in nonclinical species. Using the hepatocyte system where the full range of hepatic metabolic enzymes are represented, it appeared that the metabolism of BIC was extensive in monkey and dog but lower in rat and human.

Table 23. Metabolites of BIC Detected In Cyropreserved Hepatocytes from Different Species

Analyte[a]	Identity	Fraction of Radiochromatogram (%)			
		Wister-Han Rat	Beagle Dog	CynomolgusMonkey	Human
BIC	Parent	91.5	78.7	52.4	93.9
M305	N-dealkylation	1.7	8.7	2.4	1.2
M465a	Hydroxylation-1	1.2	1.4	2.7	-
M465b	Hydroxylation-2	-	0.2	11.6	0.6
M465c	Hydroxylation-3	-	3.6	-	-
M611	Glucose conjugation	-	0.8	4.4	-
M625	Glucuronide conjugation	5.2	6.6	21.7	4.3
M641	Hydroxylation/glucuronidation	-	-	4.1	-
Total	-	99.6	100	99.3	100

a Analyte metabolite identification numbers correspond to their molecular weight, eg, M305 = metabolite with 305 Da molecular weight
Source: AD-141-2288

5.1.2. FTC

An in vitro metabolism study was performed to identify the potential human CYP enzyme(s) responsible for the metabolism of FTC using human liver microsomes and Bactosomes containing cDNA-expressed human CYP enzymes (m2.6.5, Section 9.2.1, 15396v1). The results showed that FTC was relatively stable in the incubation medium. One minor metabolite (~1%) was detected only in incubations with cDNA-expressed CYP3A4 incubations. It was not formed by CYP1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, or 2C19. Human hepatic microsomal incubations in the presence and absence of selective inhibitors of various CYPs confirmed the low rate of FTC metabolism, and due to incomplete inhibition by the CYP3A-selective inhibitor, ketoconazole, also suggested the possible involvement of FMOs in the metabolism of FTC. In vitro glucuronidation of FTC was not detected.

Pharmacological activation of FTC involves metabolism to FTC-triphosphate, which is a direct binding inhibitor of viral polymerases. The active triphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, and ε and mitochondrial DNA polymerase γ {Flint 2003}.

5.1.3. TAF and TFV

Stability of TAF was assessed in plasma, intestinal S9, and hepatic S9 fractions from dogs and humans (m2.6.5, Section 9.3, AD-120-2023, AD-120-2024, AD-120-2025, and AD-120-2027). Tenofovir alafenamide was moderately stable in plasma and intestinal S9 with half-lives of 74.7 and 58.3 minutes for human, and 69.5 and 47.1 minutes for dog, respectively (m2.6.5, Section 9.3, AD-120-2025 and AD-120-2024). The stability of TAF in human intestinal S9 fractions was also determined in a separate study assessing the effect of HIV-PIs on TAF stability in intestinal S9 (discussed in Section 7.3.2) and a somewhat lower but similar half-life

for TAF was observed (24.5 minutes; AD-120-2027). Relative to plasma or intestinal S9, TAF was somewhat less stable in human and dog hepatic S9 fractions with half-lives of 20.6 and 31.1 minutes, respectively (Table 24). Based on these data, predicted hepatic extraction ratios for human and dog were calculated to be 76.2% and 60.5%, respectively (m2.6.5, Section 9.3.2, AD-120-2023).

Table 24. Stability of TAF in Biological Matricies from Dog and Human

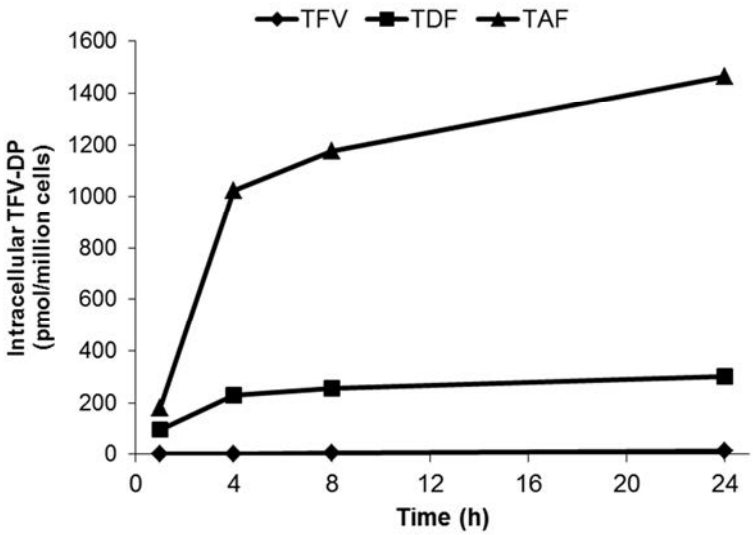
	TAF Stability, $t_{½}$ (min)		
Species	**Plasma**	**Intestinal S9**	**Hepatic S9**
Human	74.7	24.5-58.3	20.6
Beagle Dog	69.5	47.1	31.1

The potential for CYP enzymes to metabolize TAF was assessed by incubating TAF with 6 individual bacterially-expressed human CYP enzyme preparations (Bactosomes) coexpressed with human NADPH CYP reductase (m2.6.5, Section 9.3.4, AD-120-2004). Metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. Tenofovir alafenamide was slowly metabolized by CYP3A4 at a rate of 1.9 min^{-1}, which was 26.6% of the positive control, testosterone.

TAF is primarily hydrolyzed by CES1 in primary hepatocytes as described in Section 7.3.2 {Birkus 2008, Birkus 2007b, Murakami 2015}, while cathepsin A (CatA) is the major enzyme hydrolyzing TAF to TFV in PBMCs or other HIV-target cells. Tenofovir is then further phosphorylated to TFV-DP by cellular nucleotide kinases. These steps are high capacity and low affinity and are not readily inhibited by other xenobiotics.

The in vitro activation of TAF in human primary hepatocytes was evaluated and compared with that of TDF and TFV (m2.6.5, Section 9.3.5, AD-120-2017). Following a 24-hour continuous incubation of primary hepatocytes with 5 µM TAF, TDF, or TFV, the levels of TFV-DP increased to 1,470, 302, and 12.1 pmol/million cells illustrating that incubation with TAF resulted in 5- and 120-fold higher intracellular levels of TFV-DP compared to TDF and TFV, respectively (Figure 11). In primary human hepatocytes, the half-life of intracellular TFV-DP was estimated to be greater than 24 hours {Murakami 2015}.

Figure 11. Intracellular Metabolism of TAF, TDF, and TFV in Primary Human Hepatocytes



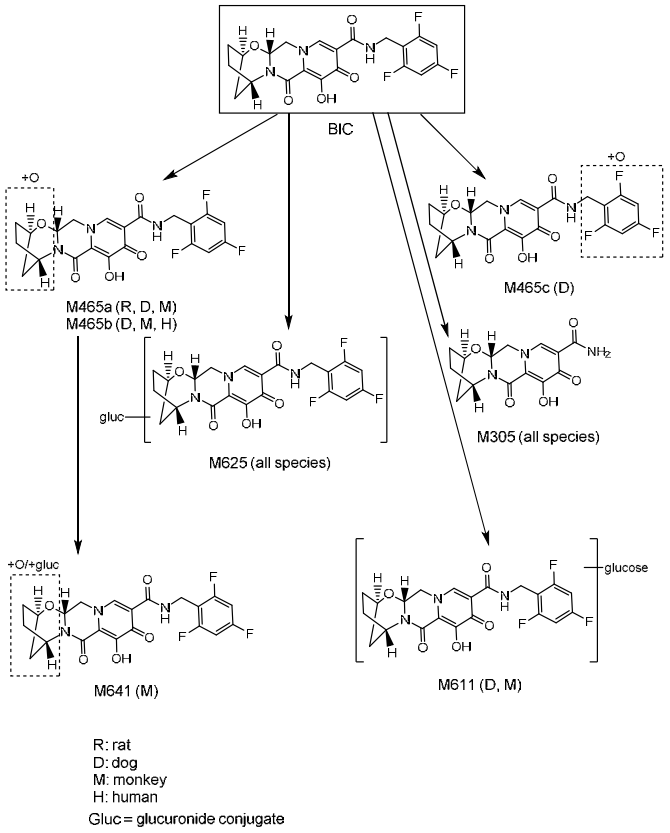
The in vitro metabolism of [^{14}C]TFV was studied in dog plasma, in control and induced (Aroclor™ 1254) rat liver microsomes, and also in dog liver and intestinal S9 fractions (m2.6.5, Section 9.3.6, 96-DDM-1278-003). Potential isomerization of TFV was determined using a chiral HPLC assay with radioactive flow detection. Radioactivity associated with the protein pellet was also determined by sample oxidation and liquid scintillation (< 0.1% of radioactivity). Tenofovir was recovered unchanged under all conditions: no metabolites were detected in either rat microsomal preparation, with or without the addition of NADPH cofactor. There was no evidence of chiral inversion. Similarly, there was no apparent loss of TFV following incubation with dog plasma, liver, or intestinal S9 fractions, and no metabolites were detected.

Summaries of the metabolic pathways for BIC, TFC, and TAF are provided in Figure 12, Figure 13, and Figure 14, respectively.

5.2. Proposed Metabolic Pathways

5.2.1. BIC

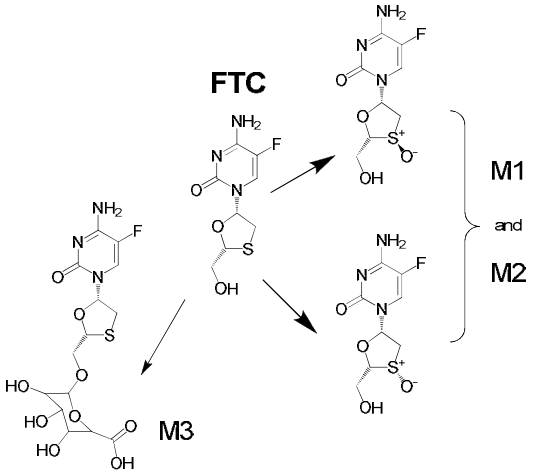
Figure 12. Proposed Identities of BIC Metabolites Identified In Vitro



5.2.2. FTC

Emtricitabine demonstrates high metabolic stability in vitro and in vivo and is the major analyte found in all samples. The metabolites of FTC detected in vitro and in vivo are illustrated in Figure 13.

Figure 13. Pathways for Metabolism of FTC Identified In Vitro and In Vivo

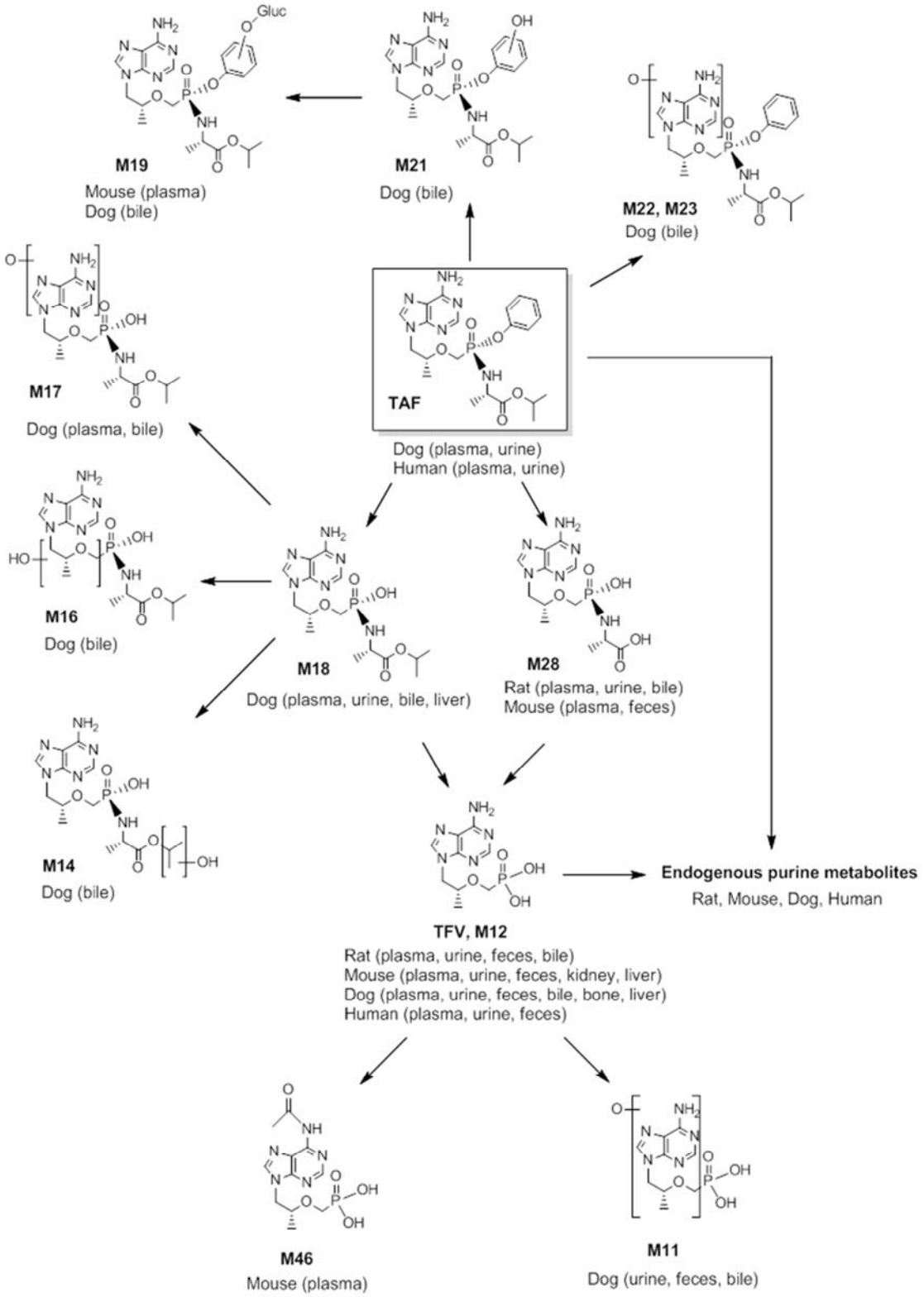


M1 and M2 are diastereomeric sulfoxide metabolites and M3 is a glucuronide conjugate

5.2.3. TAF

The metabolic profiles of TAF were determined in plasma, urine, feces, kidney, liver, and nasal turbinate from mice (m2.6.5, Section 8.3.1, AD-120-2012), in plasma, urine, bile, and feces from rats (m2.6.5, Section 8.3.2, AD-120-2021), and in plasma, urine, bile, feces, bone, and liver from dogs (m2.6.5, Section 8.3.3, AD-120-2008). The metabolite profiles were also determined in human plasma, urine, and feces following administration of a single oral dose of [^{14}C]TAF (GS-US-120-0109). Based on the results from mouse, rat, dog, and human, a proposed biotransformation pathway is summarized (Figure 14). Tenofovir alafenamide is also subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, tenofovir-monophosphate (TFV-MP) and TFV-DP (see Table 3) with TFV-DP being the pharmacologically active form.

Figure 14. Metabolites of TAF



5.3. Metabolism In Vivo

5.3.1. BIC

Bictegravir metabolism was determined following a single oral administration of [^{14}C]BIC to mouse, rat, monkey, and human. Pooled plasma, urine, bile, and fecal samples obtained following in vivo oral administration of [^{14}C]BIC were profiled and a comprehensive listing of the identified metabolites are provided in m2.6.5 in transgenic mice (m2.6.5, Section 8.1.1, AD-141-2304), intact and BDC Wistar-Han rats (m2.6.5, Section 8.1.3, AD-141-2277), intact and BDC monkeys (m2.6.5, Section 8.1.5, AD-141-2299), and healthy human subjects (GS-US-141-1481). The combined results demonstrate that BIC is mainly eliminated by hepatic metabolism followed by excretion into feces and urine. Metabolic pathways included hydroxylation, oxidative defluorination, direct glucuronidation, and oxidation followed by phase II conjugation. In the monkey, BIC was metabolized through the oxidative pathways to a greater extent compared to rat and human.

5.3.1.1. Plasma

The radiolabeled components observed in AUC pooled plasma are summarized in Table 25 and a scheme of the metabolite profile in plasma of different species is shown in Figure 15. Unchanged BIC was the most abundant circulating component in all species and as a % of total radioactivity accounted for approximately 96% in transgenic mice, 77% in rats, 80% in monkeys, and 68% in human subjects. Metabolite M20 (Figure 15), a sulfate conjugate of the hydroxylated BIC, was the most abundant metabolite in human (20.1%). M20 was also the most abundant circulating metabolite in rat and was present in monkey but at much lower abundance. Metabolite M15, a glucuronide conjugate of BIC, was the next most abundant in human (8.6%) and was also observed in monkeys. The major circulating metabolite in monkey was M42, a hydroxylated BIC (isomer of M21), unique to monkey and was not present in mouse, rat or human plasma.

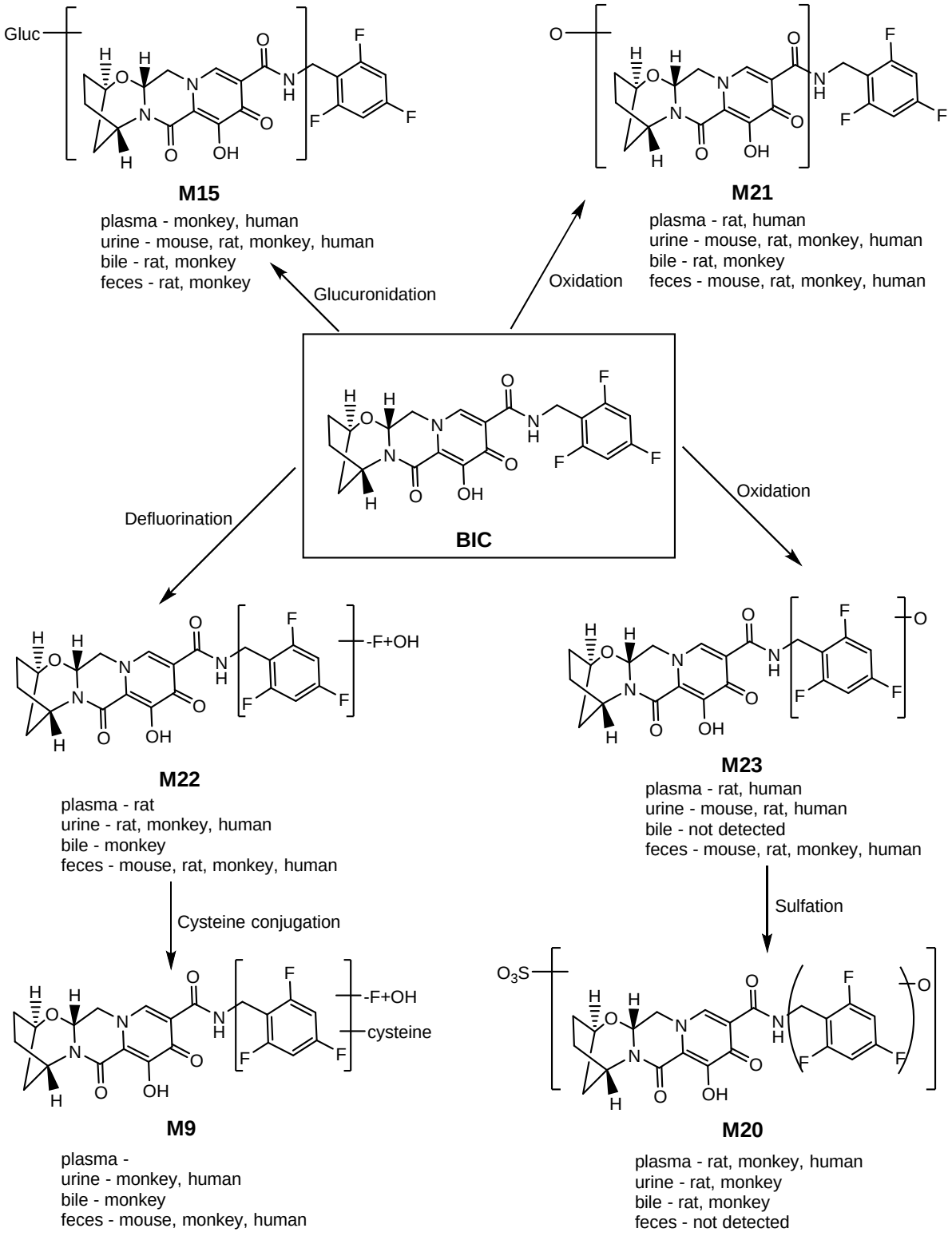
M20, the sulfate conjugate of hydroxylated BIC, was the only metabolite in human plasma greater than 10% of drug related material. Since this secondary metabolite of BIC was a polar conjugate no further assessment was required per the International Council for Harmonisation (ICH) guideline and the 2016 Food and Drug Administration (FDA) Guidance {U.S. Department of Health and Human Services Food and Drug Administration 2016}.

Table 25. Plasma Profile Following Oral Administration of [^{14}C]BIC

Component	% of Total Radioactivity in AUC Pooled Plasma[a]			
	Transgenic Mouse	Wistar Han Rat	Cynomolgus Monkey	Human
BIC	95.5	76.5	80.2	67.9
M12	1.86	2.18	ND	ND
M15	ND	ND	0.55	8.6
M20	ND	11.3	0.77	20.1
M21/M22	ND	1.18	ND	2.0
M23	ND	2.36	ND	0.2[c]
M42	ND	ND	12.2	ND
Other[b]	0.64	2.36	3.44	0.6
Total	98.0	95.9	97.2	99.4

ND = not detected
a AUC pool plasma = area under the plasma ^{14}C concentration-time curve from time zero to 48 hours post dose in transgenic mice, from time zero to 168 hours post dose in rats, from time zero to 72 hours post dose in monkeys, and from time zero to 72 hours post dose in human subjects
b Other = sum of other metabolites; each component < 1% in mouse; < 1.5% in rat, monkey, and human
c Co-eluted with M51
Source: AD-141-2304, AD-141-2077, AD-141-2299, and GS-US-141-1481

Figure 15. Proposed Biotransformation Pathways of BIC Following Oral Administration of [^{14}C]BIC



5.3.2. FTC

5.3.2.1. Mice

The metabolism and elimination of orally administered [^{3}H]FTC was studied in male CD-1 mice (m2.6.5, Section 8.2.1, TEIN/93/0015). Urine and feces were assayed by LSC and HPLC. Urinary recovery of radioactivity was 67% ± 7% of the dose. In the feces, 18% ± 3% of the dose was recovered, all as unchanged FTC. Total recovery of radioactivity excreted in urine and feces was 85% ± 4% of dose. In the urine, 64% ± 7% of the radioactivity was recovered as unchanged FTC in the 0- to 24-hour sample. Three metabolites of FTC were measurable in the urine. These metabolites were tentatively identified as: M1 and M2 (2 isomeric, 3'-sulfoxides of FTC, 1.7% ± 0.3% and 2.0% ± 0.4% of dose recovered, respectively); and 5-fluorocytosine (1.4% ± 0.1% of dose recovered). Traces of 5 other metabolites and a peak tentatively identified as tritiated water were also observed at levels of less than 1% of dose. 5-Fluorouracil was not observed (< 0.1% of dose).

5.3.2.2. Rats

The urine collected from male Sprague-Dawley rats following IV or oral administration of 10 mg/kg [^{3}H]FTC was subject to radiochromatographic profiling {Frick 1993}. Metabolite profiles were independent of the route of administration and revealed that FTC accounted for the majority of the radioactivity, with the sulfoxides (M1 and M2) being the most abundant metabolites. Small amounts were accounted for by the glucuronide (M3), 5-fluorocytosine, and tritiated water. 5-Fluorouracil was not detected (< 0.1% of the dose).

5.3.2.3. Monkeys

An exploratory study was performed with [^{3}H]FTC administered orally to female cynomolgus monkeys (m2.6.5, Section 8.2.3, TEIN/93/0016). By 72 hours postdose, 41% of radioactivity was recovered in urine, 33% in feces, and 10% in cage washings. Radiochromatographic analysis revealed that parent FTC accounted for the majority of the radioactivity in all samples (64% of urinary radioactivity and 98% of fecal radioactivity). A sulfoxide metabolite (M1 or M2) represented the majority of nonFTC radioactivity in urine and totaled 11% of the dose.

A further in vivo metabolism study was performed in cynomolgus monkeys after oral administration of [^{14}C]FTC (m2.6.5, Section 8.2.2, TOX063). Results were very similar to the previous study. Parent FTC accounted for the majority of the radioactivity in urine (74%) and feces (97%). The majority of the nonFTC radiolabel in urine was accounted for by metabolites M1, M2, and M3. Another potential urinary metabolite, 5-fluorouracil, was not detectable (< 0.02% of dose).

5.3.3. TAF and TFV

Based on the studies from mouse, rat, dog, and human (m2.6.5, Section 8.3, AD-120-2008, AD-120-2012, AD-120-2021; and GS-US-120-0109), relative quantification of TAF metabolites in plasma as percent total AUC and in urine, feces, and bile as percent total dose recovered in different species is summarized in Table 26. Endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species. Tenofovir

accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma, in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. M18 was the major metabolite in rat bile accounting for 63% of total radioactivity recovered in bile. M18 and its oxidized metabolite, M16 were the major metabolites in dog bile and accounted for 29 and 38% of total radioactivity recovered in bile, respectively. Various oxidative metabolites were found in dog bile. No metabolites unique to human were observed.

Tenofovir alafenamide-related metabolites were also monitored in kidney, liver, and nasal turbinate from mice (m2.6.5, Section 8.3.1, AD-120-2012). Most of the radioactivity was associated with TFV in kidney and liver, and xanthine (M7) was the major identified metabolite in nasal turbinates. In dog, TAF-related metabolites were monitored in bone and liver and most of the radioactivity in these tissues was associated with TFV (m2.6.5, Section 8.3.3, AD-120-2008).

M18 (isopropylalaninyl TFV) and M28 (alaninyl TFV) are considered to be intermediate metabolites during intracellular conversion of TAF to TFV. In the metabolite profiling study in dog, M28 was not detected in this study although it has been qualitatively detected previously in dog plasma at 15 minutes post dose {Babusis 2013}. It is possible that M28 may be formed transiently at low levels. M18 was detected as a minor metabolite in plasma, urine, and liver. Relatively high levels of M18 were observed in bile. Low levels of M28 were observed in rat and mouse plasma with relatively high levels in rat bile.

Table 26. Relative quantification of TAF Metabolites in Plasma, Urine, Feces, and Bile as % Total Dose Quantified

		% Total Dose			
		Plasma[a]	**Urine**	**Feces**	**Bile**
Mouse	TFV (M12)	54.8	18.1	30.7	NA[b]
	M28	1.02	0	0.7	NA
	Allantoin (M27A)	12.2	2.6	0.4	NA
	Uric acid (M27B)	19.4	0	0	NA
	Unknown metabolites	12.3	3.2	0	NA
	Total	100	23.9	31.8	NA
Rat	TFV (M12)	66.7	17.1	63.6	NA
	M28	5.8	0	0	NA
	Allantoin (M27A)	23.2	0.1	0	NA
	Unknown metabolites	4.3	1.6	0	NA
	Total	100	18.8	63.3	NA
Rat (BDC[c])	TFV (M12)	NA	17.1	61.7	0.66
	M28	NA	0.4	0	1.17
	Allantoin (M27A)	NA	0.2	0	0
	Uric acid (M27B)	NA	0	0	0.02
	Unknown metabolites	NA	1.7	0	0
	Total	NA	19.4	61.7	1.85

		% Total Dose			
		Plasma[a]	**Urine**	**Feces**	**Bile**
Dog	TAF	1.3	1.3	0	NA
	TFV (M12)	68.3	24.2	20.8	NA
	M11	0	0.4	0.4	NA
	M17	0.44	0	0	NA
	M18	17.6	0.2	0	NA
	M20	0.2	0	0	NA
	Unknown metabolites	12.2	3.0	0.2	NA
	Total	100	29.1	21.4	NA
Dog (BDC)	TAF	NA	1.3	0	0.2
	TFV (M12)	NA	16.8	26.4	1.0
	M11	NA	0.4	0.7	0.1
	M14	NA	0	0	0.2
	M16	NA	0	0	4.4
	M17	NA	0	0	0.5
	M18	NA	0	0	3.4
	M19	NA	0	0	0.1
	M21	NA	0	0	0.4
	M22	NA	0	0	0.2
	M23	NA	0.2	0	0.2
	Unknown metabolites	NA	2.9	0	0.9
	Total	NA	21.6	27.1	11.6
Human	TAF	1.8	1.41	0	NA
	TFV (M12)	1.5	22.2	31.4	NA
	Uric acid (M27B)	73.9	1.93	0	NA
	Adenine/xanthine/hypoxantine (M33, M7.M8)	0.2	0.26	0	NA
	Unknown metabolites	0	0	0.29	NA
	Total	77.4[d]	25.8	31.7	NA

a Plasma data represent % of total AUC.
b NA = not applicable
c BDC = bile duct cannulated
d Not 100% due to loss of radioactivity during sample preparations.

5.3.3.1. TFV: Monkeys

The kinetics of intracellular TFV anabolism in PBMCs, red blood cells (RBCs), and lymph nodes were studied in monkeys that received a single dose of either 15, 30, or 60 mg/kg of [^{14}C]TFV subcutaneously (m2.6.5, Section 8.3.4, P2001025). Tenofovir was efficiently taken up by PBMCs and anabolized to TFV-DP, with intracellular concentrations of the active antiviral anabolite reaching 1.6 μM (60 mg/kg dose group). The half-life of TFV-DP in this experiment was > 50 hours. Similar concentrations of TFV anabolites were observed in RBCs. Significant intracellular concentrations of TFV and its anabolites were observed in axillary, inguinal, and mesenteric lymph nodes. This long intracellular half-life of the active diphosphate form observed both in vitro and in vivo supports the proposed once daily clinical dosing regimen.

5.4. B/F/TAF

No nonclinical studies have been completed assessing the metabolism of the 3-drug combination of BIC, FTC, and TAF because each agent has distinct metabolic and excretion pathways. BIC is metabolized by CYP3A mediated oxidation and conjugation by UGT enzymes, FTC is cleared by renal excretion and TAF is metabolized to TFV by hydrolysis.

6. EXCRETION

6.1. BIC

6.1.1. Studies in Intact Mice, Rats and Monkeys and Bile Duct-Cannulated Rats and Monkeys

The excretion of radioactivity was determined following a single oral administration of [^{14}C]BIC to the male mouse, rat, and monkey. A comprehensive listing of the extent, routes, and rates of excretion are provided in m2.6.5 — transgenic mice (m2.6.5, Section 12.1.1, AD-141-2303); intact and BDC Wistar Han rats (m2.6.5, Sections 12.1.2 and 13.1.2, AD-141-2276); and intact and BDC cynomolgus monkeys (m2.6.5, Sections 12.1.3 and 13.1.3, AD-141-2298). The cumulative excretion over a 1-week collection period is summarized in Table 27. The average cumulative overall recovery of dosed radioactivity was > 80% in all species studied. The excretion routes in intact animals were consistent across species, with the majority of the excreted dose in feces (> 40% of dose) and with minor amounts in urine (< 21% of dose). The excretion into bile in BDC rat and monkey was approximately 34% to 40% of dose, respectively. In combination with metabolite profiling, these results demonstrate that BIC was mainly eliminated through metabolism by the liver followed by excretion into feces and urine.

Table 27. Cumulative Dose Recovery Following a Single Oral Administration of [^{14}C]BIC to Male Transgenic Mice at 2 mg/kg (300 µCi/kg, n=4), Intact and BDC Wistar Han Rats at 2 mg/kg (100 µCi/kg, n=3), and Intact and BDC Cynomolgus Monkeys at 1 mg/kg (25 µCi/kg, n=3)

Species	Collection Time (h)	Cumulative Recovery of Radioactivity (% Dose)				
		Urine	Feces	Bile	Carcass (residual)	Total Excreta[a]
Mice	0–24	3.21	89.9	NA	NA	NA
	0–48	3.48	96.6	NA	NA	NA
	0–168	3.55	98.5	NA	0.0799	102
Intact WH Rats	0–24	1.95 ± 0.39	22.9 ± 1.1	NA	NA	NA
	0–48	3.16 ± 0.70	41.8 ± 4.5	NA	NA	NA
	0–168	5.01 ± 0.84	76.3 ± 3.5	NA	13.7 ± 4.2	95.9 ± 0.4
BDC WH Rats	0–24	3.15 ± 0.60	9.97 ± 1.64	13.0 ± 5.4	NA	NA
	0–48	4.65 ± 0.91	21.0 ± 2.2	19.6 ± 6.1	NA	NA
	0–168	7.48 ± 1.19	42.4 ± 4.7	34.1 ± 5.1	13.7 ± 5.5	99.1 ± 1.0
Intact Cynomolgus Monkeys	0–24	17.8 ± 2.6	10.7 ± 5.2	NA	NA	NA
	0–48	19.7 ± 3.1	21.7 ± 11.6	NA	NA	NA
	0–168	20.8 ± 3.3	40.9 ± 3.7	NA	NA	80.4 ± 7.8
BDC Cynomolgus Monkeys	0–24	13.5 ± 5.1	5.09 ± 2.14	38.2 ± 7.3	NA	NA
	0–48	14.6 ± 5.0	16.7 ± 6.1	39.4 ± 7.6	NA	NA
	0–168	15.2 ± 5.0	20.3 ± 6.5	39.7 ± 7.7	NA	86.0 ± 1.7

BDC = bile duct cannulated; NA = not applicable; WH = Wistar Han
a Total recovery includes radioactivity in excreta carcass, cage rinses, cage wash, cage wipe, cage debris, bile cannula rinse, and jacket rinse.
Source: AD-141-2303, AD-141-2276, and AD-141-2298

6.2. FTC

6.2.1. Excretion of Radioactivity after Administration of [^{3}H]FTC to Mice

Male CD-1 mice were dosed orally with 120 mg/kg [^{3}H]FTC (m2.6.5, Section 8.2.1, TEIN/93/0015). Recovery of radioactivity up to 72 hours postdose totaled 85.0% ± 4.2% of the dose, with 66.8% ± 7.0% in urine and 18.1% ± 3.1% in feces. The majority of the radioactivity was excreted during the first collection period (0–24 hours postdose), with 62.3% ± 7.6% of the dose in urine and 15.9% ± 3.0% of the dose in feces.

6.2.2. Excretion of Radioactivity after Administration of [^{3}H]FTC to Rats

Male Sprague-Dawley rats were dosed intravenously or orally with 10 mg/kg [^{3}H]FTC and urine and feces collected for 6 days {Frick 1993}. After IV administration, recovery of radiolabel in excreta was 96% ± 3.7%, with 91% ± 3.4% in urine and 5.0% ± 1.6% in feces. Results were similar following oral dosing, with total recovery of 99% ± 3.2% and 74% ± 2.8% in urine and 25% ± 1.6% in feces.

6.2.3. Excretion of Radioactivity after Administration of FTC to Monkeys

Following oral administration of 80 mg/kg [^{3}H]FTC to female cynomolgus monkeys, an average of 83.8% ± 3.8% of dosed radioactivity was recovered by 72 hours postdose, with 41.2% ± 6.4% of the dose in urine, 33.1% ± 10.0% in feces, and 9.6% ± 6.7% in cage washings (m2.6.5, Section 8.2.3, TEIN/93/0016). The majority of the radioactivity was recovered in the first collection phase (32.9% ± 8.6% in the 0–8 hour urine sample and 23.6% ± 15.9% in the 0–24 hour feces sample).

In a second study, an oral dose of 200 mg/kg [^{14}C]FTC was given to male cynomolgus monkeys and urine and feces were collected up to 120 hours postdose (m2.6.5, Section 8.2.2, TOX063). The excretion pattern was similar to the previous study: total recovery of radioactivity averaged 76.2% ± 4.1% of the dose, with 40.8% ± 8.5% of the dose in urine and 35.4% ± 8.6% in feces. However, excretion of radiolabel took longer than the previous study, with cumulative recovery of radiolabel in urine of 15.1% ± 5.1% by 12 hours postdose and 24.8% ± 7.3% by 24 hours postdose.

6.3. TAF/TFV

6.3.1. Excretion of Radioactivity after Administration of [^{14}C]TAF to Mice

After oral administration of 100 mg/kg [^{14}C]TAF to CD-1 mice, most of the radioactivity was eliminated by 48 hours postdose (m2.6.5, Section 5.3.1, AD-120-2011). An average of approximately 61% of the radioactive dose was recovered in urine and feces from CD-1 mice through 48 hours postdose. A large amount of radioactivity (average of 6.65% of the dose) was recovered in the cage rinse. An average of 41.3 and 27.7% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. An average overall recovery of radioactivity after oral dosing to CD-1 mice was 83.2%.

6.3.2. Excretion of Radioactivity after Administration of [^{14}C]TAF or [^{14}C]TFV to Rats

The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male Sprague-Dawley rats (m2.6.5, Section 5.3.2, AD-120-2020). The results from BDC rats are discussed in Section 6.3.4. Most of radioactivity derived from [^{14}C]TAF was rapidly excreted within 24 hours after oral dosing. The mean values of 71.9 and 22.2% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. The mean overall recovery of radioactivity was 96.7%.

The effect of dose on excretion of [^{14}C]TFV was evaluated in Sprague-Dawley rats following IV administration at doses of 10 or 50 mg/kg (400 µCi/kg) (m2.6.5, Section 12.3.1, 96-DDM-1278-001). Following dosing at 10 mg/kg, the mean cumulative recovery in the urine/cage wash was 85.2% ± 7.63 % by 24 hours and 92.7% ± 6.77 % by 7 days postdose. The mean terminal elimination half-life calculated from urine data was 15.82 ± 1.79 hours. The mean recovery of the administered dose in the feces was 3.18% ± 1.85% by 24 hours, and 4.48% ± 1.89% by 7 days postdose. Similar results were seen following dosing at 50 mg/kg. Tenofovir was the only species present in the urine and feces; no metabolites were detected. These results indicate that TFV is primarily excreted by renal clearance of the unchanged drug.

6.3.3. Excretion of Radioactivity after Administration of [^{14}C]TAF or [^{14}C]TFV to Dogs

The excretion of [^{14}C]TAF was determined after administration of a single 15-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male dogs (m2.6.5, Section 13.2.1, AD-120-2007). The results from BDC dogs are discussed in Section 6.3.4. Radioactivity derived from [^{14}C]TAF was readily excreted mostly within 48 hours after oral dosing. The mean values of 37.4% and 35.9% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. Overall mean recovery of radioactivity was 80.4%.

Tenofovir excretion was also evaluated following IV administration of [^{14}C]TFV (m2.6.5, Section 13.2.2, 96-DDM-1278-002). The primary route of elimination was via the kidneys, as 70.03% of the total radioactive dose was recovered in the urine during the first 48 hours following dosing. Total fecal recovery of radioactivity was 0.42% of the total dose.

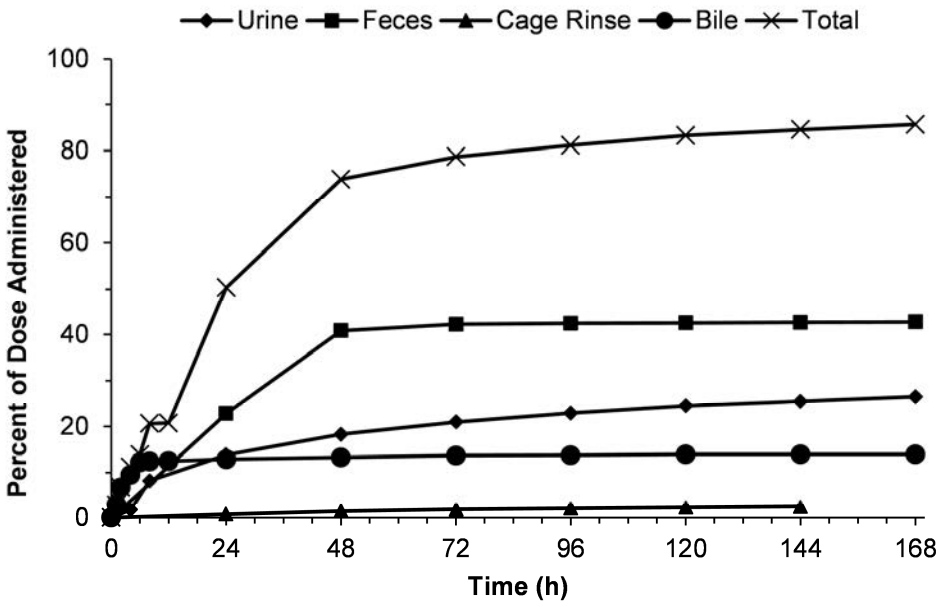
6.3.4. Excretion into Rat and Dog Bile

The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to BDC male Sprague-Dawley rats (m2.6.5, Section 5.3.2, AD-120-2020). Mean values of 72.6%, 23.2%, and 2.11% of the administered radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. Recoveries of radioactivity in bile and urine from BDC rats indicated that at least 25% of the dose was absorbed. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%.

The excretion of [^{14}C]TAF was determined following oral administration of a single 15-mg/kg dose of [^{14}C]TAF to male BDC dogs (m2.6.5, Section 13.2.1, AD-120-2007). Mean values of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and

bile, respectively, through 168 hours postdose (Figure 16). Based on the radioactivity excreted in urine and bile, a minimum of approximately 41% of the orally administered dose was absorbed. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. The overall recovery of radioactivity in BDC dogs was 86.2%. Radioactivity was measurable in urine and feces at 168 hours postdose, indicating low recoveries were probably due to radioactivity retained in the carcasses.

Figure 16. Cumulative Percent Total Radioactive Dose Recovered in Urine, Feces, Bile, and Cage Rinse Following Oral Administration of [^{14}C]TAF at 5 mg/kg to Bile Duct-Cannulated Dogs



The extent of biliary excretion of radioactivity following a single IV administration of 10 mg/kg [^{14}C]TFV to a beagle dog was evaluated (m2.6.5, Section 13.2.2, 96-DDM-1278-002). Total biliary recovery of radioactivity through 48 hours postdose was 0.26% of the total dose.

6.3.5. Excretion into Milk

Milk was obtained from 2 lactating adult female rhesus monkeys following a single 30 mg/kg subcutaneous dose of TFV (m2.6.5, Section 7.3.2.2, P2000116). Both milk and serum samples were collected over time up to 24 hours postdose. Concentrations of TFV in milk reached an apparent maximum at 4 hours for 1 animal and at 1 hour in the second. Tenofovir C_{max} in milk was 4.04% and 2.02% of the observed C_{max} in plasma for the 2 animals, respectively, and declined with apparent half-lives of 10.3 and 10.9 hours. The TFV AUC in milk was 18.6% and 21.5% of the observed AUC in plasma for the 2 animals, respectively.

6.4. B/F/TAF

No nonclinical excretion studies have been done with the combination of BIC, FTC, and TAF. BIC is metabolized by CYP3A mediated oxidation and conjugation by UGT enzymes and then eliminated into bile and then into feces. FTC is eliminated primarily intact by renal excretion. TAF is metabolized by hydrolysis to TFV and is then eliminated by renal excretion. Since BIC, FTC, and TAF have distinct metabolic and excretion pathways for elimination, the coadministration of BIC, FTC, and TAF is not anticipated to change the excretion of the individual compounds.

7. PHARMACOKINETIC DRUG INTERACTIONS

Discussions of drug interaction liability are made by reference to current industry and United States and European regulatory guidelines {Bjornsson 2003, European Medicines Agency 2012, Giacomini 2010, U.S Department of Health and Human Services (DHHS) 2012}.

7.1. BIC

7.1.1. Cytochrome P450 and UGT1A1 Inhibition

The potential for BIC to reversibly inhibit major human drug metabolizing CYP enzymes was determined in human hepatic microsomal fractions with known substrates of individual enzymes (m2.6.5, Section 11.1.1, AD-141-2293). Bictegravir at 100 µM had little or no inhibitory effect on the activities of any of the CYP isoforms; the IC_{50} was > 100 µM for all CYPs. Thus BIC is unlikely to cause significant drug interactions in vivo through inhibition of human CYP enzymes based on the calculated AUC ratio (AUCR) values (< 1.2; m2.6.5, Section 14.1.7, AD-141-2313) using the FDA net effect model as well as the calculated $[I]/K_{i,u}$ (< 0.02; m2.6.5, Section 14.1.7, AD-141-2313) based on the European Medicines Agency (EMA) guidance.

Table 28. Assessment of CYP Inhibition Potential of BIC

Enzyme	Activity	% inhibition at 100 µM BIC	BIC IC_{50} (µM)
CYP1A2	Phenacetin *O*-deethylase	-0.987	>100
CYP2B6	Bupropion 4-hydroxylase	13.3	>100
CYP2C8	Paclitaxel 6α-hydroxylase	23.5	>100
CYP2C9	Tolbutamide 4-hydroxylase	40.4	>100
CYP2C19	*S*-Mephenytoin 4'-hydroxylase	42.0	>100
CYP2D6	Dextromethorphan *O*-demethylase	0.737	>100
CYP3A	Midazolam 1'-hydroxylase	34.3	>100
CYP3A	Testosterone 6β-hydroxylase	33.8	>100

CYP = cytochrome P450; IC_{50} = concentration resulting in 50% inhibition
Values are the mean of triplicate determinations
Source: AD-141-2293

The potential for BIC to be a mechanism-based inhibitor of the human CYP enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A, was assessed at a BIC concentration of 100 µM (m2.6.5, Section 11.1.3, AD-141-2308). No time-dependent inhibition was observed for BIC against CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP2D6; the maximum change in activity observed was 12.1% with CYP2D6 relative to control. Bictegravir exhibited substrate-dependent inhibition of CYP3A, with an effect on midazolam 1'-hydroxylase activity (39.8% relative to control) but no meaningful effect (< 8% relative to control) on testosterone 6β-hydroxylase activity. The effect on CYP3A activity was confirmed using the IC_{50} shift protocol, wherein a shift of ≥ 3.1 fold, from >200 µM to 64.3 µM was

observed. Collectively these data suggest that BIC is a weak (K_I > 100 μM) mechanism-based inhibitor of human CYP3A, but not of any of the other enzymes tested. Further determination of kinetic parameters was not possible due to solubility limitations and weak activity. Therefore, BIC is unlikely to be a clinically relevant mechanism-based inhibitor of CYP3A.

The potential for BIC to inhibit UGT1A1 was determined in human hepatic microsomal fractions containing alamethicin and UDP-glucuronic acid (m2.6.5, Section 11.1.2, AD-141-2294). Bictegravir was a very weak inhibitor of human UGT1A1 with a calculated IC_{50} of 176 μM.

7.1.2. Enzymology of Metabolism

The human CYP450 isoforms responsible for CYP-mediated metabolism of [^{3}H]BIC (2 μM) were identified using 8 individual recombinant expressed CYP450 enzyme preparations co-expressed with human NADPH CYP reductase (m2.6.5, Section 9.1.2, AD-141-2290). CYPs 3A4 and 3A5 were the only two isoforms that metabolized BIC; no turnover was observed with other isoforms (Table 29). The human UGT enzymes responsible for formation of the direct glucuronide (M15 in Figure 15) was identified using 12 individual recombinant expressed human UGT enzyme preparations and the rates of glucuronidation of BIC (5 μM) were determined (m2.6.5, Section 9.1.3, AD-141-2291). The recombinant human UGT1A1 formed the largest quantity of BIC-glucuronide (M15) under the conditions tested; lesser quantities of the same glucuronide were also generated by UGT1A3, 1A8 and 1A9 (Table 30). These in vitro findings were consistent with the results from a clinical DDI study in healthy subjects — administration of rifampin (a strong CYP3A4 and UGT1A1 inducer) reduced BIC plasma AUC by 75% and administration of atazanavir (an UGT1A1and CYP3A4 inhibitor) increased BIC plasma AUC by 315%; and administration of voriconazole (a strong CYP3A4 inhibitor) increased BIC plasma AUC by 61% (GS-US-141-1485).

Table 29. Rates of Generation of BIC Metabolites by Human CYP Enzymes

	[^{3}H]BIC Metabolism	
	% Formed at 45 min[b]	
Enzyme	**Met-1[a]**	**Met-2[a]**
CYP1A2	ND	ND
CYP2B6	ND	ND
CYP2C8	ND	ND
CYP2C9	ND	ND
CYP2C19	ND	ND
CYP2D6	ND	ND
CYP3A4	39	1.9
CYP3A5	55	7.0

CYP = cytochrome P450; ND = not detected
a Met-1 and Met-2 = metabolites observed in the radiochromatogram; structures were unassigned
b % Metabolite formation = 100% × Metabolite peak area at 45 min/Sum of all peak areas at 45 min
Source: AD-141-2290

Table 30. Rates of Formation of BIC Glucuronide by Major Human UGT Enzymes

Enzyme	BIC Glucuronide (M15) Formation (PAR × 10^{-3} at 60 min)
UGT1A1	12.0
UGT1A3	1.0
UGT1A4	ND
UGT1A6	ND
UGT1A7	ND
UGT1A8	1.0
UGT1A9	3.0
UGT1A10	ND
UGT2B4	ND
UGT2B7	ND
UGT2B15	ND
UGT2B17	ND

ND = not detected; PAR = Peak area ratio of M15 to internal standard; UGT = uridine diphosphate glucuronosyl transferase
Source: AD-141-2291

7.1.3. Assessment of Induction Liability

The potential for BIC to induce human drug metabolizing enzymes through the activation of AhR and PXR was assessed in vitro in reporter cell lines (m2.6.5, Section 11.1.4, AD-141-2292). Bictegravir (50 µM) showed little activation (< 5% of maximal effect of β-naphthoflavone) of AhR and modest activation of PXR (40% of maximal effect of rifampicin) in the respective reporter cell assays.

The potential for BIC to induce CYP enzymes, UGT1A1, and P-gp was assessed in cultured human hepatocytes from 3 different donors (m2.6.5, Section 11.1.5, AD-141-2305) and the results are summarized in Table 31 and Table 32. Bictegravir (1 – 60 µM) treatment led to no significant increases (< 2-fold) in mRNA of CYPs 2C8 and 2C9 or mRNA and activity of CYP1A2. Bictegravir was a very weak inducer of CYP2B6 as concentration dependent mRNA increases were observed with a 4.74 fold increase at 60 µM BIC; however no increase in CYP2B6 activity was observed in any of the donors.

Bictegravir was a weak inducer of CYP3A4 − concentration dependent mRNA increases of up to 16.7 fold at 60 µM BIC were observed, whereas, only small increases (up to 2.6-fold) in CYP3A activity were observed. As the calculated AUCR values for CYP3A4 crossed the threshold of AUCR < 0.8 (m2.6.5, Section 14.1.7, AD-141-2313), a clinical study was conducted. The plasma PK of the CYP3A4 sensitive substrate midazolam and partial substrates velpatasvir and voxilaprevir were unaffected by coadministration with B/F/TAF FDC in clinical DDI studies (GS-US-380-4270 and GS-US-380-1999). Repeat dose administration of BIC resulted in no

change in the plasma elimination phase half-life of BIC, suggesting a lack of autoinduction (GS-US-141-1218). Further, repeat dose administration of BIC did not affect norgestimate and ethinyl estradiol PK (GS-US-311-1790). The cumulative data indicate that BIC following oral dosing is unlikely to be a clinically relevant inducer of CYP3A.

P-glycoprotein mRNA increased 5.76-fold at a 60 μM BIC concentration with no increase observed at lower BIC concentrations. However, repeat dosing of BIC in humans did not reduce the plasma exposure of a P-gp sensitive substrate TAF (GS-US-141-1218). In contrast, repeat dose administration of known P-gp inducers had a discernible effect on TAF plasma PK; carbamazepine (a strong P-gp inducer) decreased TAF plasma AUC and C_{max} by approximately 55% (GS-US-311-1387); and efavirenz (a moderate P-gp inducer) decreased TAF plasma AUC and C_{max} by 14% and 22%, respectively (GS-US-311-0101).

Table 31. Effect of BIC Treatment on CYP Activity in Cultured Human Hepatocytes

Treatment	Mean Fold Increase of CYP Activity over Vehicle Control (%positive control)		
	CYP1A2	CYP2B6	CYP3A4
BIC (1 μM)	1.00 (0.00%)	0.95 (-0.45%)	1.19 (2.24%)
BIC (3 μM)	1.01 (0.068%)	1.09 (0.82%)	1.36 (4.24%)
BIC (10 μM)	0.86 (-0.96%)	1.15 (1.36%)	2.50 (17.7%)
BIC (30 μM)	0.63 (-2.53%)	0.76 (-2.18%)	2.57 (18.5%)
BIC (60 μM)	0.42 (-3.97%)	0.73 (-2.45%)	1.73 (8.60%)
Omeprazole (50 μM)	15.6	NA	NA
Phenobarbital (1000 μM)	NA	12.0	NA
Rifampin (10 μM)	NA	NA	9.49

CYP = cytochrome P450; NA= not applicable
Probe substrates were phenacetin, bupropion, and testosterone for CYP1A2, 2B6, and 3A, respectively.
Data represent the mean from 3 donors
Source: AD-141-2305

Table 32. Effect of BIC Treatment on mRNA Levels in Cultured Human Hepatocytes

Treatment	Mean mRNA Fold Increase over Vehicle Control (%positive control)						
	CYP1A2	CYP2B6	CYP3A4	CYP2C8	CYP2C9	UGT1A1	P-gp
BIC (1 μM)	1.10 (0.61%)	1.19 (1.81%)	1.71 (3.13%)	1.62 (67.4%)	1.07 (36.8%)	1.18 (1.80%)	1.02 (2.27%)
BIC (3 μM)	1.11 (0.67%)	1.63 (6.00%)	2.50 (6.61%)	1.67 (72.8%)	1.18 (94.7%)	1.22 (2.20%)	0.87 (-14.8%)
BIC (10 μM)	1.22 (1.35%)	2.41 (13.4%)	7.20 (27.3%)	1.98 (107%)	1.26 (137%)	1.89 (8.90%)	1.00 (0.00%)
BIC (30 μM)	1.18 (1.10%)	3.93 (27.9%)	16.4 (67.8%)	1.78 (84.8%)	1.20 (105%)	3.55 (25.5%)	1.38 (43.2%)
BIC (60 μM)	0.74 (-1.60%)	4.74 (35.6%)	16.7 (69.2%)	1.10 (10.9%)	1.37 (195%)	3.51 (25.1%)	5.76 (541%)
EC_{50}[a] (μM)	NA	103	19.1	NA	NA	143	NA
Omeprazole (50 μM)	17.3	NA	NA	NA	NA	11.0	NA
Phenobarbital (1000 μM)	NA	11.5	NA	NA	NA	NA	NA
Rifampin (10 μM)	NA	NA	23.7	1.92	1.19	NA	1.88

CYP = cytochrome P450; NA = not applicable; P-gp = P-glycoprotein; UGT = uridine diphosphate glucuronosyl transferase
a Values were extrapolated by curve fitting (constrained to E_{max} of 100%)
Data are the mean from 3 donors
Source: AD-141-2305

7.1.4. Interactions with Transporters

The potential for BIC to be a substrate of human efflux and uptake transporters was determined in transfected cellular systems (m2.6.5, Section 14.1.1, AD-141-2278, and Section 14.1.2, AD-141-2275) and the results are summarized in Table 33. Bictegravir was a substrate for human P-gp and BCRP transporters. These results are consistent with efflux transport observed in Caco-2 cells (Section 3.1.1). Bictegravir (1 μM) was not a substrate of OATP1B1 or OATP1B3 uptake transporters.

Table 33. Permeability of BIC (10 μM) in Wild Type, Pgp- or BCRP-Overexpressing MDCKII Cells

P_{app} ($\times 10^{-6}$ cm/sec) of BIC	Wild Type MDCKII	P-gp-overexpressing MDCKII		BCRP-overexpressing MDCKII	
		- inhibitor	+ inhibitor[a]	- inhibitor	+ inhibitor[a]
Forward (A to B)	18.6	6.3	12.5	8.1	19.0
Reverse (B to A)	23.3	47.6	30.3	52.3	38.7
Efflux Ratio	1.3	7.5	2.4	6.5	2.0

BCRP = breast cancer resistance protein; BIC = bictegravir (GS-9883); MDCKII = Madine-Darby canine kidney cell line; P-gp = P-glycoprotein; P_{app} = apparent permeability coefficient
a Control inhibitor: P-gp, cyclosporin A (10 μM); BCRP, Ko134 (10 μM)
Source: AD-141-2278

The potential for BIC to inhibit human drug transporters was determined in cell lines transfected with individual transporters or using membrane vesicle preparations (m2.6.5, Section 14.1.3, AD-141-2273, Section 14.1.4, AD-141-2274, Section 14.1.5, AD-141-2285, and Section 14.1.6, AD-141-2310) and the results are summarized in Table 34. Bictegravir did not inhibit OATP1B1, OATP1B3, or OAT1 mediated transport. Bictegravir, at the highest concentration tested (80 or 100 µM), weakly inhibited P-gp (20%), BCRP (6%), BSEP (46%), OCT1 (13%), and OAT3 (64%). Bictegravir showed dose-dependent inhibition of MATE1with an IC_{50} value of 8.0 µM. Bictegravir was an inhibitor of the renal uptake transporter OCT2 in vitro, with an IC_{50} value of 0.42 µM. A clinical PK and PD DDI study (GS-US-380-3908) was conducted with B/F/TAF and metformin (an OCT2 and MATE1 substrate). Metformin plasma exposure was increased approximately 39% following coadministration with B/F/TAF, relative to placebo; however, the PD characteristics of metformin, such as glucose metabolism, and active GLP-1 and lactate levels after OGTT, were unaffected by coadministration with B/F/TAF.

Table 34. Inhibition Potential of Transporters by BIC

Transporter	Maximum Inhibition at Highest Concentration Tested (Concentration)	IC_{50} (µM)	Report
P-gp	20% (80 µM)	>80	AD-141-2273
BCRP	6% (80 µM)	>80	AD-141-2273
BSEP	46% (100 µM)	>100	AD-141-2310
OATP1B1	No inhibition (80 µM)	>80	AD-141-2274
OATP1B3	No inhibition (80 µM)	>80	AD-141-2274
OCT1	13% (100 µM)	>100	AD-141-2310
OCT2	94% (10 µM)	0.42	AD-141-2285
OAT1	No inhibition (100 µM)	>100	AD-141-2310
OAT3	64% (100 µM)	55	AD-141-2310
MATE1	79% (80 µM)	8.0	AD-141-2285

BCRP = breast cancer resistance protein; BSEP = bile salt export pump; IC_{50} = concentration resulting in 50% inhibition; MATE = multidrug and toxin extrusion protein; OAT = organic anion transporter; OATP = organic anion-transporting polypeptide; OCT = organic cation transporter; P-gp = P-glycoprotein

7.2. FTC

In all species examined, FTC shows high oral bioavailability, low plasma binding, and is eliminated, largely unchanged, by renal excretion. Metabolism by CYP3A (and possibly FMO) enzymes plays a minor role in FTC clearance. It is thus unlikely that FTC will be a victim of drug interactions, due to inhibition or induction of drug metabolizing enzymes or drug transporters at the intestine or liver. It is also unlikely that FTC would affect the metabolism of coadministered medications through inhibition or induction and clinical experience with FTC to date supports this conclusion. Consistent with the low induction potential, FTC did not activate human AhR or human PXR up to 50 µM in vitro (m2.6.5, Section 11.2.2, AD-162-2005). FTC was a substrate of human OAT3 and was not a substrate of human OAT1 (m2.6.5, Section 14.4.2, AD-236-2010).

7.3. TAF and TFV

The PK profiles of TAF and TFV following oral administration of TAF are described in Table 35. The unbound C_{max} of TAF was calculated based on the percent unbound value of 20% obtained from multiple human ex vivo studies; this should be more clinically relevant than the value determined in vitro, which had somewhat higher percent unbound TAF.

Table 35. Steady State Pharmacokinetic Parameters for TAF and TFV

	TAF	TFV
Dose (mg)	25	-
Total C_{max} (µM)	0.37	0.060
Unbound C_{max} (µM)[a]	0.075	0.060
Intestinal (µM)[b]	210	-
$C_{hep, inlet}$ [c]	0.72	-

a Calculated based on percent unbound values of 20% for TAF (GS-US-120-0108 and GS-US-120-0114) and 99.3% for TFV (Section 4.1.3)
b Estimated based on TAF 25 mg dose.
c Estimated total hepatic inlet (portal vein) concentration calculated based on TAF 25 mg dose according to Obach et al. {Obach 2006} Absorption rate constant of 0.01 min^{-1} and human hepatic blood flow of 1500 mL/min were used for estimation.

The potential of TAF to be a victim or perpetrator of DDIs was assessed in various in vitro systems. The potential of TAF or its metabolites to inhibit CYP enzymes and UGT1A1 or induce CYP enzymes, UGT1A1, or P-gp and serve as substrates or inhibitors of xenobiotic transporters was assessed. The effect of other drugs, including other antiviral agents that may be coadministered with TAF, on intestinal stability and the absorption potential was also determined.

7.3.1. Cytochrome P450 and UGT1A1 Inhibition

The potential for TAF and TFV to inhibit human CYP-mediated drug metabolism was examined in vitro using hepatic microsomal fractions and enzyme-selective activities (m2.6.5, Section 11.3.1, AD-120-2003 and Section 11.3.2, V990172-104). The inhibitory activity of TAF with human liver microsomal CYP isozymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A were assessed at concentrations up to 25 µM. The inhibition constant (IC_{50}) values calculated for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 were greater than 25 µM. Tenofovir alafenamide weakly inhibited CYP3A-mediated oxidation of midazolam or testosterone with IC_{50} of 7.6 or 7.4 µM, respectively. However, the weak inhibition of CYP3A is not clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or RPV in clinical DDI studies (GS-US-120-1538 and GS-US-120-1554). Tenofovir at 100 µM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A.

The potential for TAF to be a mechanism-based inhibitor of the human CYP enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A was assessed at a TAF concentration of 50 µM (m2.6.5, Section 11.3.3, AD-120-2040). There was no evidence for time- or cofactor-dependent inhibition of any enzyme by TAF and the maximum change in activity observed for any CYP was 17.4% with CYP2C8 relative to control.

Tenofovir alafenamide was assessed for inhibition of estradiol 3-glucuronide formation in insect cell microsomal fractions containing baculovirus-expressed human UGT1A1 (m2.6.5, Section 11.3.6, AD-120-2006). Tenofovir alafenamide did not inhibit UGT1A1 up to 50 µM (IC_{50} > 50 µM).

7.3.2. Enzymology of Metabolism

Orally administered TAF undergoes intestinal absorption. During intestinal absorption, TAF may be metabolized by intestinal esterases and/or CYP enzymes. The effects of HIV-1 PIs and CYP inhibitors on the stability of TAF in intestinal subcellular fractions were determined (m2.6.5, Section 9.3.7, AD-120-2027). Incubation of TAF with the HIV-1 PIs atazanavir or darunavir, or the CYP inhibitors, ritonavir or COBI did not markedly affect the stability of TAF in intestinal subcellular fractions at concentrations up to 100 µM (Table 36).

Table 36. Stability of TAF in Human Intestinal Subcellular Fraction in the Absence and Presence of Test Compounds

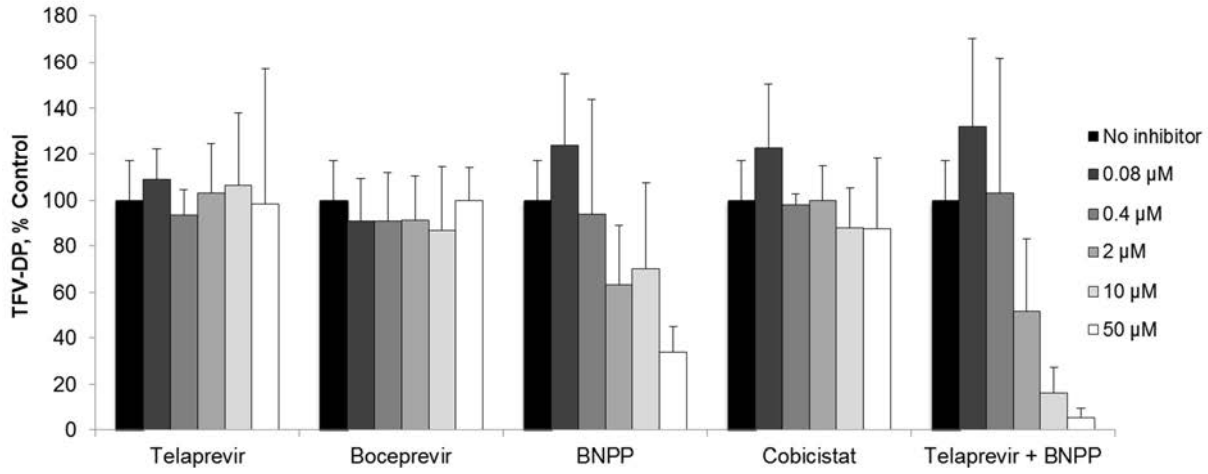
Inhibitor	Concentration (µM)	Intestinal S9 Stability[a] $t_{1/2}$ (min)
Vehicle control	0	24.5 ± 4.1
Atazanavir	25	28.9 ± 5.2
	100	38.9 ± 5.3
Darunavir	25	32.2 ± 5.1
	100	30.8 ± 5.0
Ritonavir	25	19.0 ± 2.8
	100	18.9 ± 1.5
Cobicistat	25	30.1 ± 5.1
	100	32.9 ± 4.8
Dichlorvos	500	> 789[b]

a Data are mean ± SD, n = 2 (6 data points per replicate)
b Less than 10% loss of substrate in 120 minutes

To understand the enzymes involved in activation of TAF in primary human hepatocytes, cells were incubated with TAF together with known CatA inhibitors (approved hepatitis C virus nonstructural protein 3 (NS3) inhibitors, telaprevir and boceprevir), CES1 inhibitor (bis-p-nitrophenyl phosphate [BNPP]), CYP3A4 and P-gp inhibitor (COBI), or telaprevir and BNPP together (m2.6.5, Section 9.3.8, AD-120-2031). As shown in Figure 17, the metabolism of TAF was inhibited by BNPP in a dose-dependent manner. Little or no effect on TFV-DP formation was observed with telaprevir, boceprevir, or COBI. When telaprevir and BNPP were combined a greater inhibition was seen at higher concentrations of the 2 compounds. These results indicated that TAF is primarily hydrolyzed by CES1 in primary human hepatocytes with CatA also making a contribution. Several CES1 genetic variants that are associated with the enzyme activity have been identified at very low frequency; G143E (heterozygous: 2%-4% and homozygous: 0.05%) and D260fs (very rare) {Tarkiainen 2012, Zhu 2008}. In a study of HBV-infected subjects, a total of 42/51 (82.4%) patients were genotyped for the rs71647871 (G143E) variant of CES1. All patients were found to carry the reference homozygous genotype, ie, there

were no carriers of the minor allele of this variant genotype. In addition, since TAF can be activated by both CES1 and CatA in liver, the genetic polymorphisms causing a marked effect on TAF activation should be extremely rare.

Figure 17. Effects of Esterase and CYP inhibitors on Intracellular Activation of TAF in Primary Human Hepatocytes



7.3.3. Assessment of Induction Liability

The potential for TAF to induce human drug metabolizing enzymes and drug transporters through the activation of human AhR or human PXR was evaluated in cell-based systems (m2.6.5, Section 11.3.4, AD-120-2005). For PXR activation, at 50 µM TAF the extent of activation of PXR was only 23% of the maximal effect of rifampicin and 15 µM TAF demonstrated activation of < 5% of the maximal induction elicited by rifampicin. Tenofovir alafenamide did not activate AhR up to 50 µM, the highest concentration tested. Therefore, TAF is unlikely to activate either of these human xenobiotic receptors.

The induction of CYP, P-gp, and UGT1A1 mRNA and CYP activity by TAF was assessed in cultured human hepatocytes from 3 separate donors treated with 1, 10, and 100 µM TAF added once daily for 3 consecutive days (m2.6.5, Section 11.3.5, AD-120-2032). CYP induction data as mean fold increase in mRNA levels and activity upon treatment with TAF and corresponding positive controls are summarized in Table 37. Due to cytotoxicity, the cell viability was significantly affected at 100 µM TAF and mixed responses to TAF with increased mRNA levels and reduced CYP activities were observed. At noncytotoxic concentrations of TAF (1 and 10 µM), no significant increases in the mRNA levels and the CYP activities were observed. After treatment with 10 µM TAF, the mRNA levels of CYP1A2 and CYP3A4 increased by 3.0- and 8.3-fold which correspond to 3% and 6% of the induction levels observed with the respective positive controls. Therefore, TAF showed little or no potential for CYP induction at clinically relevant concentration (1 µM). No significant induction of P-gp and UGT1A1 mRNA was observed (less than 2-fold). Furthermore, TAF is unlikely to be a clinically relevant inducer as it did not affect the exposure to midazolam or RPV (GS-US-120-1538 and GS-US-120-1554).

Table 37. Effect of TAF Treatment on CYP mRNA Levels and Activity in Cultured Human Hepatocytes (mean, n = 3 donors)

	Mean Fold Increase (% Positive Control)					
	mRNA			Activity[c]		
Concentration	CYP1A2	CYP2B6	CYP3A	CYP1A2	CYP2B6	CYP3A
1 µM TAF	1.2 (<1%)	0.95 (<1%)	0.92 (<1%)	1.0 (<1%)	1.1 (<1%)	0.97 (<1%)
10 µM TAF	3.0 (3%)	1.6 (4%)	8.3 (6%)	1.4 (1%)	0.85 (<1%)	0.99 (<1%)
100 µM TAF[a]	6.9 (8%)	2.5 (10%)	44 (36%)	0.84 (<1%)	0.42 (<1%)	0.37 (<1%)
Positive control[b]	72	16	120	28	13	29

a The viability of the hepatocytes was affected at this concentration of TAF and therefore caution should be taken when interpreting the corresponding induction data.
b Positive controls 50 µM omeprazole, 1000 µM phenobarbital, and 10 µM rifampin for CYP1A2, CYP2B6, and CYP3A, respectively.
c Phenacetin, bupropion, and testosterone were used as probe substrates for CYP1A2, 2B6, and 3A, respectively.

7.3.4. Potential for Transporter-Mediated Drug Interactions with TAF and TFV

The potential for TAF and TFV to inhibit or to act as substrates for drug transporters has been assessed in vitro. Inhibition constants and substrate assessments for tested transporters are summarized Table 38. Tenofovir alafenamide showed little or no inhibition of the transport of model substrates by P-gp, BCRP, OAT1, OAT3, and OCT2 (m2.6.5, Section 14.3.2, AD-120-2019 and Section 14.3.6, AD-120-2036). Weak inhibition of OATP1B1, OATP1B3, BSEP, OCT1, and MATE1 was observed but none of these transporters were inhibited by 50% at 100 µM TAF, which is approximately 200-fold over the total maximal plasma concentrations. Therefore, TAF is unlikely to be a perpetrator of transporter-mediated drug interactions.

The route of elimination of TFV is renal excretion by a combination of glomerular filtration and tubular secretion. In order to understand the role of transporters in the renal secretion of TFV and to explore potential drug interactions based on these transport systems, the interactions of TFV with a variety of both uptake and efflux transporters were studied in vitro.

Results of in vitro transport studies indicate that the active tubular secretion of TFV is mediated by human OAT1 (basolateral uptake) and MRP4 (apical efflux) transporters acting in series in proximal tubules (m2.6.5, Section 14.3, PC-103-2001, AD-104-2001, AD-104-2002) {Cihlar 2004, Cihlar 2001, Ray 2005}. Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP2 appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, OAT1 has been assessed for its potential role in drug interactions between TFV and other therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including PIs). Under physiologically relevant conditions, none of the tested drugs affected OAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV due to inhibition of this pathway (m2.6.5, Section 14.3.9, PC-104-2010 and Section 14.3.10, PC-104-2011) {Cihlar 2001}. Unlike TFV, TAF was not a substrate for renal transporters, OAT1 and OAT3.

The results from in vitro studies investigating the contribution from MRP1 in tubular reabsorption of TFV (m2.6.5, Section 14.3.11, PC-104-2014) indicated that MRP1 is not involved in the reabsorption of TFV at the basolateral membrane of proximal tubule cells.

Tenofovir did not inhibit the activity of human OCT2 or MATE1 (IC_{50} > 300 µM) so TFV is unlikely to cause drug interactions through inhibition of these transporters (m2.6.5, Section 14.3.12, AD-104-2012).

Table 38. Transporter Substrate and Inhibition Assessment of TAF and TFV

Transporter	Substrate Potential (y/n)		Inhibition Potential, IC_{50} (µM)		m2.6.5 Section, Report
	TAF	TFV	TAF	TFV	
P-gp	y	n	>100	>1000	Section 14.3.1, AD-120-2018 Section 14.3.16, AD-236-2004 Section 14.3.2, AD-120-2019 Section 14.3.15, AD-236-2003
BCRP	y	n	>100	>100	Section 14.3.1, AD-120-2018 Section 14.3.17, AD-236-2005 Section 14.3.2, AD-120-2019 Section 14.3.15, AD-236-2003
BSEP	ND	ND	>100	>100	Section 14.3.20, AD-236-2008 Section 14.3.6, AD-120-2036
OATP1B1	y	ND	>100	>100	Section 14.3.4, AD-120-2022 Section 14.3.2, AD-120-2019 Section 14.3.18, AD-236-2006
OATP1B3	y	ND	>100	>100	Section 14.3.4, AD-120-2022 Section 14.3.2, AD-120-2019 Section 14.3.18, AD-236-2006
MATE1	ND	ND	>100	>300	Section 14.3.6, AD-120-2036 Section 14.3.12, AD-104-2012
OAT1	n	y	>100	33.8[a]	m2.6.3, Section 1.6, PC-120-2018 Section 14.3.9, PC-104-2010 Section 14.3.6, AD-120-2036 Section 14.3.19, AD-236-2007
OAT3	n	y	>100	>1000	m2.6.3, Section 1.6, PC-120-2018 Section 14.3.10, PC-104-2011 Section 14.3.6, AD-120-2036 Section 14.3.19, AD-236-2007 Section 14.3.13, PC-103-2001
OCT1	n	n	>100	>100	Section 14.3.6, AD-120-2036 Section 14.3.13, PC-103-2001 Section 14.3.20, AD-236-2008
OCT2	ND	n	>100	>300	Section 14.3.21, AD-236-2011 Section 14.3.6, AD-120-2036 Section 14.3.13, PC-103-2001
MRP1	ND	n	ND	>500	Section 14.3.11, PC-104-2014
MRP2	ND	n	ND	>100	Section 14.3.7, AD-104-2001
MRP4	ND	y	ND	>1000[b]	Section 14.3.7, AD-104-2001 Section 14.3.19, AD-236-2007

BCRP = breast cancer resistance protein; BSEP = bile salt excretory pump; MATE = multidrug and toxin extrusion protein; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = organic cation transporter 1

a Binding constant for uptake into CHO cells reported by Cihlar et al, 2009 {Cihlar 2001}.

b Imaoka et al 2007 {Imaoka 2007}

Tenofovir alafenamide is a substrate for intestinal efflux transporters, P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors, CsA or COBI in vitro (m2.6.5, Section 3.3.1, AD-120-2037 and m2.6.5, Section 14.3.3, AD-120-2013). Cobicistat is a weak inhibitor of intestinal efflux transporters, but high concentrations of COBI in the intestinal lumen, achievable briefly during absorption, may inhibit P-gp and result in increase in TAF exposure. As shown in Table 39, in the presence of 90 µM COBI in the Caco-2 bidirectional permeability assay, TAF forward permeability increased 4.6-fold and the efflux ratio significantly decreased, suggesting an efflux transporter mediated drug interaction (m2.6.5, Section 14.3.3, AD-120-2013). The effect of CsA on TAF oral bioavailability was also assessed in vivo in dogs (m2.6.5, Section 14.3.14, AD-120-2035). As described in Table 40, plasma PK parameters were determined in dogs following oral administration of TAF at 2 mg/kg to untreated or pretreated animals with 75 mg CsA. The CsA pretreatment increased the plasma exposure to TAF and oral bioavailability by approximately 10-fold, while the PK profile of TFV was slightly increased by CsA. Consistent with the increased TAF plasma exposure, the exposure to TFV-DP in PBMCs isolated from the CsA pretreated dogs was approximately 2-fold higher than that in cells from untreated animals. These results suggest that coadministration of efflux inhibitors increases TAF absorption.

Table 39. Effect of COBI on the Bidirectional Permeability of TAF in Caco-2 Cells

Inhibitor	Direction	TAF Initial Concentration (µM)	Recovery (%)	P_{app} ($\times 10^{-6}$ cm/s)	Efflux Ratio
None	Cell-Free	9.61	119	30.8	—
	Forward	9.92	64	0.74	20
	Reverse	8.71	102	15.1	
COBI (90 µM)	Forward	11.0	101	3.4	1.6
	Reverse	11.4	115	4.9	

Table 40. Mean Plasma Pharmacokinetic Parameters for TAF and TFV Following Oral Administration of TAF to Male Beagle Dogs

	No CsA Pretreatment		Pretreatment with 75 mg CsA	
Parameter	TAF	TFV	TAF	TFV
AUC_{0-t} (nM•h)	31.4	1140	312	1330
T_{max} (h)	0.14	0.667	0.24	1.0
C_{max} (nM)	109	342	813	187
$t_{1/2}$ (h)	0.21	>24	0.15	>24
%F	1.67	NA	16.6	NA

NA = Not applicable

Tenofovir alafenamide was efficiently taken up and metabolized in primary human hepatocytes. As shown in Figure 18, TAF was taken up by untransfected CHO cells at a rate of 9.0 pmol/min/10^6 cells indicating that TAF has high passive permeability. Uptake was higher with the cells expressing hepatic uptake transporter, OATP1B1 or OATP1B3 with rates of 12.0 or 24.1 pmol/min/10^6 cells, respectively and rifampin inhibited the transporter dependent uptake. Atorvastatin and antipyrine were used as positive and passive permeability controls, respectively. These results demonstrated that TAF is a substrate for hepatic uptake transporters, OATP1B1 and OATP1B3 (m2.6.5, Section 14.3.4, AD-120-2022). Effect of an OATP inhibitor, rifampicin on uptake of TAF into primary human hepatocytes was assessed in vitro. As shown in Figure 19, the results from four different hepatocyte donors suggested that OATP-mediated transport makes a small contribution to TAF uptake (m2.6.5, Section 14.3.5, AD-120-2042). Taken together, it is likely that the major route of TAF uptake into hepatocytes is passive permeability. Therefore, while exposure to TAF may be affected slightly by inhibitors of these transporters or genetic polymorphisms that affect the transport activities, the effects of differences in OATP1B1 and OATP1B3 activity are, not expected to be clinically relevant given the high passive permeability of TAF.

Figure 18. OATP1B1- and OATP1B3-Mediated Uptake of TAF

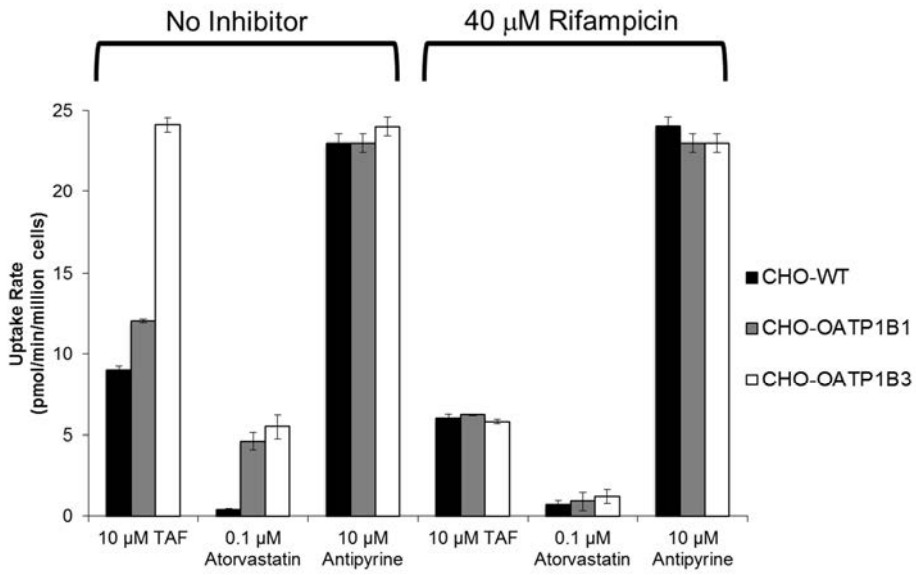
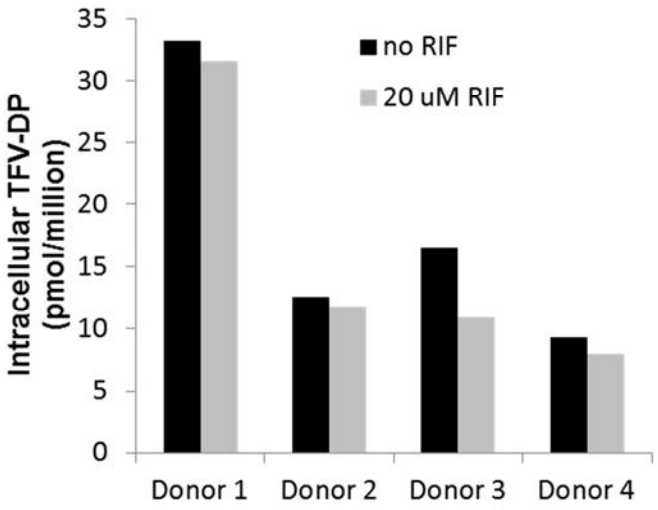


Figure 19. Effect of Rifampicin on Uptake of TAF into Primary Human Hepatocytes



7.4. B/F/TAF

No nonclinical DDI studies have been done with the combination of BIC, FTC, and TAF.

8. OTHER PHARMACOKINETIC STUDIES

There are no additional studies to report.

9. DISCUSSION AND CONCLUSIONS

Comprehensive nonclinical studies have been carried out on BIC, FTC, and TAF as individual agents to assess their absorption, distribution, metabolism, elimination and drug interaction potentials. Results from these nonclinical studies are discussed below.

9.1. BIC

Absorption, distribution, metabolism, and excretion studies support the selection of rat, rabbit, and monkey as nonclinical species for the toxicology assessment of BIC. The metabolism and elimination profiles of BIC in nonclinical species were consistent with those observed in humans during clinical studies. Following oral administration of BIC in all species studied, unchanged parent drug was the predominant circulating component in plasma. All human metabolites were also found in nonclinical species. Biliary or renal excretion of parent drug is a minor route of elimination of BIC.

High permeability and efflux transport were observed in vitro and evidence for moderate to high fractional absorption was observed in rat, dog, and monkey. The oral bioavailability of BIC ranged from 42% to 74% in nonclinical species. Bictegravir was highly protein bound (> 98%) with a volume of distribution of lower than total body water in rat, dog, and monkey. Bictegravir showed minimal partition into erythrocytes with a blood to plasma ratio close to 0.6 in all species. Bictegravir was a substrate for efflux transporters and poorly crossed the blood-brain barrier in rats.

Bictegravir was a substrate of CYP3A and UGT1A1. Oxidation, direct glucuronidation, and oxidation followed by phase II conjugation were the major metabolic pathways for BIC in rats, monkeys, and humans. Unchanged BIC was the major circulating species observed in mice, rats, monkeys, and humans. Bictegravir was mainly eliminated by hepatic metabolism followed by excretion into feces and urine. Since CYP3A and UGT1A1 play a major role in the elimination of BIC, the systemic exposure of BIC may be altered by inducers or inhibitors of these enzymes.

Bictegravir, at concentrations up to 100 μM, did not reversibly inhibit the metabolic functions of CYP enzymes, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4, or UGT1A1 in human hepatic microsomal fractions. Bictegravir was a weak mechanism based inhibitor of CYP3A. A weak induction by BIC of drug metabolizing enzymes or transporters regulated by PXR and constitutive androstane receptor (CAR) was observed in primary human hepatocytes. Bictegravir is highly bound to plasma proteins (human > 99%) and is therefore unlikely to be a perpetrator of drug interactions through induction or inhibition of UGT1A1 or CYP enzymes. The low potential for clinically meaningful DDIs was confirmed in dedicated clinical studies; the plasma PK of midazolam, a sensitive CYP3A4 substrate, was unaffected by coadministration with B/F/TAF FDC.

Bictegravir was a substrate for intestinal efflux transporters P-gp and BCRP and its intestinal absorption may be decreased by inducers or increased by coadministered inhibitors of P-gp and BCRP. Bictegravir was not an inhibitor of the hepatic transporters OATP1B1, OATP1B3, OCT1, or BSEP, or the renal transporters OAT1 and OAT3 at clinically relevant concentrations. Although BIC was an inhibitor of renal transporters OCT2 and MATE1, clinical studies with B/F/TAF FDC showed a lack of clinically relevant change in the plasma AUC of metformin with no change in the PD characteristics of metformin.

9.2. FTC

Emtricitabine is rapidly and extensively absorbed after oral administration in mice, rats, and cynomolgus monkeys, with oral bioavailability ranging from 58% to 97%. Exposure is roughly dose-proportional over most of the range explored. Therefore absorption of FTC is likely rapid and complete in humans over the clinical dose range. Emtricitabine is not extensively metabolized; therefore its bioavailability is likely to be governed only by absorption, with little or no first-pass metabolism in the intestinal wall or liver.

Studies of FTC in mouse, rabbit, monkey, and human plasma show that FTC has little or no measurable binding (≤ 3.6%). The observed volume of distribution for FTC (~ 0.9 L/kg) is dose-independent and close to that of total body water and suggests free distribution of FTC between tissue (intracellular and extracellular) and plasma. The low level of protein binding for FTC also suggests that drug interactions due to altered protein binding will not occur for this drug. Since FTC is largely excreted unchanged and does not bind significantly to plasma proteins, the renal clearance of FTC should be similar to its total body clearance. Emtricitabine clearance values generally exceeded the glomerular filtration rate and approach renal plasma flow, suggesting that the kidneys not only filter FTC passively, but also actively secrete it in urine, a phenomenon observed with other pyrimidine nucleosides {Frick 1993}.

Emtricitabine is widely distributed in the body. After oral administration, the highest concentrations are found in the intestine and kidneys, consistent with its absorption and elimination via these tissues. Levels in CNS tissues reach ~ 2% to 9% of those in plasma.

In radiolabel studies in mice, rats, and cynomolgus monkeys, metabolism accounts for only a minor percentage of FTC elimination. Only trace levels of metabolites were found in feces. Over 90% of the radioactivity in mouse and rat urine, and 64% of the radioactivity in monkey urine was unchanged drug. The principal metabolite was a 3'-sulfoxide (M1 or M2), accounting for approximately 2% of the dose in mice, 2.6% in rats, and 6% to 11% in monkeys. Other metabolites were detected in the urine of rats and cynomolgus monkeys, but none accounted for more than 2% of the dose. These minor metabolites may include 5-fluorocytosine, the other diastereomeric 3'-sulfoxide, and a glucuronide conjugate (M3). No 5-fluorouracil was detected in any samples. Thus, in contrast to other nucleoside analogues, FTC is not extensively metabolized and is eliminated primarily as unchanged drug by renal excretion. Based on these observations, it is unlikely that FTC will be subject to significant first-pass metabolism, or to changes in clearance due to hepatic disease or metabolic drug interactions.

The biotransformation of FTC in humans is similar to that in monkeys, yielding the same 3 putative metabolites (oxidation of the thiol moiety to form M1 and M2 and conjugation with glucuronic acid to form M3). These metabolites were only quantifiable at low levels in urine samples. Metabolite M2 was the most predominant, with its urinary recovery accounting for 8.7% of the dose administered. M2 was sporadically quantifiable in plasma samples of 2 out of 5 subjects and when measurable, plasma M2 concentrations were 30- to 50-fold lower than plasma FTC concentrations at corresponding time points. Urinary recoveries of metabolites M1 and M3 accounted for only 0.3% and 4% of the dose administered, respectively. These 3 metabolites along with unchanged FTC in urine accounted for essentially the entire dose recovered in urine (~86%). Emtricitabine does not inhibit human CYP and demonstrates no liability to be an inducer.

9.3. TAF

Oral administration of TAF generates sufficient exposure to TAF and/or TFV in nonclinical species chosen for assessment of toxicology. Consistent with dose-dependent permeability observed in vitro, the oral bioavailability of TAF increased with increasing dose in dogs and the observed oral bioavailability was 14.3% at the 10-mg/kg dose {Babusis 2013}. Following a 15-mg/kg oral dose of [^{14}C]TAF to BDC dog, the fraction absorbed was at least 41% based on excretion in urine and bile. Therefore, hepatic extraction was calculated to be approximately 65%, which was consistent with that estimated from the in vitro stability study in dog hepatic S9 fractions (60.5%). High levels of pharmacologically active TFV-DP were observed in dog liver following oral administration of TAF and persisted with an apparent half-life of > 20 hours. In vitro, high levels of intracellular TFV-DP were observed following incubation of primary human hepatocytes with TAF with 5- and 120-fold higher levels compared to incubation with TDF and TFV, respectively. Consistent with the long half-life of TFV-DP in dog liver, the half-life of intracellular TFV-DP was estimated to be greater than 24 hours {Murakami 2015}.

Following oral administration of [^{14}C]TAF to mouse, rat, and dog, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver; high levels of radioactivity were also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse. No melanin binding was observed in rats. TAF poorly penetrates into CSF following oral administration in monkeys.

The biotransformation of TAF was studied in mice, rats, and dogs and compared with that in humans. Endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species including humans. Tenofovir accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. No metabolites unique to human were observed. In addition, none of the intermediate metabolites formed during intracellular conversion of TAF to TFV (eg, M18 [isopropylalaninyl TFV] and M28 [alaninyl TFV]), were observed in humans. The major enzymes involved in intracellular conversion of TAF to TFV in primary human hepatocytes and PBMCs are CES1 and CatA, respectively. Tenofovir is further phosphorylated to pharmacologically active TFV-DP by cellular nucleotide kinases. These steps are usually high capacity and low affinity and are not readily inhibited by other xenobiotics.

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. Total recovery of radiolabel is high for all species.

Tenofovir alafenamide is not an inhibitor of UGT1A1 and CYP enzymes except for weak inhibition observed for CYP3A in vitro. While TAF is a weak inhibitor of CYP3A in vitro, it is not a clinically meaningful inhibitor of CYP3A as TAF did not affect the exposure to CYP3A

substrates, midazolam or RPV in clinical DDI studies. Tenofovir alafenamide is not a clinically relevant inducer of CYP enzymes, UGT1A1, or P-gp. Tenofovir alafenamide is unlikely to be a perpetrator of transporter-mediated drug interactions. Since TAF is a substrate for intestinal efflux transporters P-gp and BCRP, TAF exposure may be affected by inhibitors and/or inducers of the intestinal efflux transporters. While TAF is also a substrate for hepatic uptake transporters, OATP1B1 and OATP1B3, these transporters make small contributions to TAF uptake into hepatocytes and the effects of changes in the transporter activities are not expected to be clinically relevant given the high passive permeability of TAF. Tenofovir alafenamide was not a substrate for renal transporters OAT1 and OAT3 suggesting that TAF is not contributing to renal tubular cell loading of TFV; as a result, intracellular TFV concentrations in renal cells are likely to correlate with plasma TFV levels, which are 90% lower following the administration of TAF than of TDF. Overall, TAF has high hepatic extraction and is efficiently metabolized into pharmacologically active TFV-DP in the liver cells.

9.4. B/F/TAF

BIC, FTC and TAF have distinct metabolic and excretion pathways for elimination. BIC is metabolized by CYP3A mediated oxidation and conjugation by UGT enzymes and then eliminated into feces and urine. FTC is eliminated primarily intact by renal excretion. TAF is predominantly hydrolyzed intracellularly to TFV and is then eliminated by renal excretion. Based on the nonoverlapping routes of clearance and elimination, the coadministration of BIC, FTC, and TAF is not anticipated to result in a clinically relevant DDI. This was confirmed in a clinical DDI study wherein concomitant administration of BIC and F/TAF showed no significant PK DDI and no dose adjustment was necessary when BIC is administered or coformulated together with F/TAF.

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SECTION 2.6.5—PHARMACOKINETICS TABULATED SUMMARY

BICTEGRAVIR/EMTRICITABINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (B/F/TAF FDC)

Gilead Sciences

[redacted] **20**

TABLE OF CONTENTS

NOTE TO REVIEWER

This application is being submitted in support of a fixed dose combination (FDC) that contains the integrase strand-transfer inhibitor (INSTI) bictegravir (BIC, B), nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®) and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF, GS-7340) fumarate (GS-7340-03): the B/F/TAF FDC (50/200/25 mg) tablet.

All nonclinical studies to support the B/F/TAF FDC application are included with no cross-referencing to data previously submitted. This comprises all nonclinical tests utilizing BIC, FTC, or tenofovir disoproxil fumarate (TDF)/TAF, including relevant combination studies, eg FTC/TDF; and other studies necessary to support the proposed product labeling. Links to all study reports included in the dossier are highlighted in blue text.

Comprehensive programs of nonclinical studies have been conducted with BIC, FTC, and TDF/TAF as single agents. Information from all nonclinical studies with BIC, FTC, or TDF/TAF should be considered in the context of the substantial clinical experience with FTC and TDF/TAF within antiretroviral combination therapy for the treatment of human immunodeficiency virus-1 (HIV-1) infection, and experience in the Phase 2 and 3 studies in combination with BIC as BIC/FTC/TAF FDC.

The following conversions are provided to aid the reviewer:

- BIC (GS-9883) 1 μM = 0.449 μg/mL
- FTC (GS-9019) 1 μM = 0.247 μg/mL
- TAF (GS-7340) 1 μM = 0.477 μg/mL
- TFV (GS-1278; tenofovir) 1 μM = 0.287 μg/mL

1. PHARMACOKINETICS: OVERVIEW

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation (BIC)					
Mouse plasma	No	NA	In Vitro	[redacted]	BA-141-2008 (8316723)
Rat plasma	No	NA	In Vitro	[redacted]	BA-141-2001 (8292929)
Rat plasma	No	NA	In Vitro	[redacted]	BA-141-2007 (8305404)
Rabbit plasma	No	NA	In Vitro	[redacted]	BA-141-2006 (8305405)
Monkey plasma	No	NA	In Vitro	[redacted]	BA-141-2002 (8292930)
Analytical Methods and Validation (FTC)					
Mouse, monkey, human plasma, and human urine	No	NA	In Vitro	[redacted], USA	97/001.01
Mouse, rabbit, monkey plasma	No	NA	In Vitro	[redacted], USA	6159v1
Mouse, rat, human plasma	No	NA	In Vitro	[redacted], USA	6447v5
Monkey, human urine	No	NA	In Vitro	[redacted], USA	7582v1

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation (TAF)					
Mouse plasma	No	NA	In Vitro	[redacted], USA	BA-120-2004
Rat plasma	No	NA	In Vitro	[redacted], USA	BA-120-2003
Rabbit plasma	No	NA	In Vitro	[redacted], USA	BA-120-2005
Dog PBMC	No	NA	In Vitro	[redacted], Canada	993680 MYS (D990175)
Dog, monkey, human plasma	No	NA	In Vitro	[redacted], Canada	TOX-120-002 Appendix 32
Monkey plasma	No	NA	In Vitro	[redacted], Canada	BA-120-2010
Monkey plasma	No	NA	In Vitro	[redacted], Canada	BA-120-2011
Monkey PBMC	No	NA	In Vitro	[redacted], Canada	BA-120-2012
Monkey PBMC	No	NA	In Vitro	[redacted], Canada	BA-120-2013
Monkey plasma	No	NA	In Vitro	[redacted], Canada	010520/PDW
Monkey plasma	No	NA	In Vitro	[redacted], Canada	010521/PHZ
Monkey PBMC	No	NA	In Vitro	[redacted], Canada	AA01240-RQZ (P2000114)

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation (TFV)					
Mouse plasma	No	NA	In Vitro	[redacted], USA	P4331-00008 (97-TOX-4331-08, OLI-RE748-9807-DNS-1)
Rat, monkey plasma	No	NA	In Vitro	[redacted], USA	P1278-00001 (OLI-VRA144.1)
Rat milk	No	NA	In Vitro	[redacted], Canada	P1278-00034 (003105/OUI)
Rat plasma	No	NA	In Vitro	[redacted], Canada / [redacted], Canada	P1278-00028 (001097/NDK)
Rat plasma	No	NA	In Vitro	[redacted], Canada	001092/NGE
Dog plasma	No	NA	In Vitro	[redacted], USA	P1278-00017 (OLI-VRA144.2)
Dog plasma	No	NA	In Vitro	[redacted], Canada	P4331-0037 (003296/OTN)
Monkey plasma	No	NA	In Vitro	[redacted], Canada	P1278-00029 (002092/OFH)
Absorption After a Single Dose (BIC)					
Permeability across Caco-2 cell monolayers	No	Caco-2 Cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2295
Absorption	No	Mouse	Oral	[redacted]	AD-141-2307 (8311189 & 8316059)

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption	No	Rat	IV, Oral	[redacted]	AD-141-2279 (BG-0401-DA-RE)
Absorption	No	Rat	Oral	[redacted]	AD-141-2286 (BG-0463-DA-RE & BG-0505-DA-RE)
Absorption	No	Rat	Oral	[redacted]	AD-141-2296 (8295133)
Absorption	No	Rat	Oral	[redacted]	AD-141-2306 (BG-0463-DA-RE)
Absorption	No	Rabbit	Oral	[redacted]	AD-141-2300 (8266635)
Absorption	No	Dog	IV, Oral	[redacted]	AD-141-2280 (814-1436 & BG-0431-DA-DE)
Absorption	No	Cynomolgus Monkey	IV, Oral	[redacted]	AD-141-2281 (814-1445 & 814-1463)
Absorption	No	Cynomolgus Monkey	Oral	[redacted]	AD-141-2284 (8287-516 & 8287530)
Absorption	No	Cynomolgus Monkey	Oral	[redacted]	AD-141-2297 (8298452)
Absorption	No	Rhesus Monkey	IV	[redacted]	AD-141-2282 (8279078)
Absorption After a Single Dose (FTC)					
Absorption, Bioavailability	Yes	Mouse	IV, Oral	[redacted], USA	TEIN/93/0003
Absorption, Bioavailability	Yes	Mouse	IV, Oral	[redacted], USA	TEIN/93/0004

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption, Bioavailability	Yes	Mouse	IV, Oral	[redacted], Scotland	IUW00101
Absorption, Bioavailability	Yes	Cynomolgus Monkey	IV, Oral	[redacted], USA	TEZZ/93/0019
Absorption, Bioavailability	No	Cynomolgus Monkey	IV, Oral	[redacted], Scotland	IUW00301
Absorption After a Single Dose (TAF)					
Permeability across Caco-2 cell monolayers	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2037
Absorption	Yes	Mouse	Oral	[redacted], USA	AD-120-2014
Absorption	No	Mouse	Oral	[redacted], USA	AD-120-2016
Absorption	Yes	Rat	Oral	[redacted], USA	AD-120-2015
Absorption	No	Rat	Oral	Gilead Sciences, Inc., Boulder, CO, USA (In-life phase); Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	R990130
Formulation comparison	No	Rat	Oral	Gilead Sciences, Inc., Boulder, CO, USA (in-life phase); [redacted], Canada (Analysis)	R2000065
Absorption, Bioavailability	No	Dog	IV, Oral	[redacted], USA (In-life phase), Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	99-DDM-1278-001-PK

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption	No	Dog	Oral	[redacted], USA (In-life phase), Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	AD-120-2034
Absorption, Bioavailability	No	Rhesus Monkey	Oral	[redacted], USA, (In-life phase), [redacted] Canada (Analysis)	P2000087
Absorption, Bioavailability (TFV)	No	Rhesus Monkey	IV, Oral	[redacted] USA, (In-life phase), [redacted] Canada (Analysis)	P2000031 (P4331-00033)
Absorption After Repeated Dose (FTC)					
Toxicokinetics	Yes	Mouse	Oral	[redacted], USA Gilead Sciences Inc., Foster City, CA, USA	TOX-109
Toxicokinetics	No	Mouse	Oral	[redacted], Scotland	IUW00701
Toxicokinetics	No	Mouse	Oral	[redacted], USA	TOX 599
Toxicokinetics	No	Mouse	Oral	[redacted], Scotland	TOX 022
Toxicokinetics	No	Mouse	Oral	[redacted], USA	TOX 628
Toxicokinetics	Yes	Rat	Oral	[redacted] Gilead Sciences Inc., Foster City, CA, USA	TOX 108

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Toxicokinetics	No	Rat	Oral	[redacted] Gilead Sciences Inc., Foster City, CA, USA	TOX 097
Toxicokinetics	No	Cynomolgus Monkey	Oral	[redacted], USA	TOX 600
Toxicokinetics	No	Cynomolgus Monkey	Oral	[redacted], USA	TOX 627
Toxicokinetics	No	Cynomolgus Monkey	Oral	[redacted], USA	TOX 032
Absorption After Repeated Dose (TAF)					
Repeated dose pharmacokinetics	No	Dog	Oral	[redacted], USA (In-life phase) Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	AD-120-2033
Toxicokinetics	Yes	Dog	Oral	[redacted] Canada (In-life phase); [redacted], USA (Analysis); [redacted], USA (Analysis); [redacted], Canada (Analysis)	D990175-PK
Toxicokinetics	Yes	Monkey	Oral	[redacted] (In-life phase); [redacted] Canada (Analysis)	P2000114-PK

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Distribution (BIC)					
Plasma protein binding	No	Plasma (Rat, Dog, Monkey and Human)	In Vitro	[redacted] and Gilead Sciences, Inc., Foster City, CA	AD-141-2287 (60D-1333)
Microsomal binding	No	Human Liver Microsomes	In Vitro	[redacted], UK	AD-141-2311 (174-R361)
Blood plasma ratio	No	Whole Blood (Rat, Dog, Monkey and Human)	In Vitro	[redacted], UK	AD-141-2312 (174-R283 & 174-R284)
Tissue distribution of radioactivity	No	Rat (Wistar Han and Long Evans)	Oral	[redacted]	AD-141-2276 (8292819)
Distribution (FTC)					
Plasma protein binding	No	In Vitro	In Vitro	[redacted], USA	TBZZ/93/0025
Tissue distribution, Excretion	No	Rat	Oral	[redacted], USA	TOX092
Distribution (TAF)					
Plasma protein binding in vitro	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2026
Plasma protein binding in vitro of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	P0504-00039.1
Tissue distribution of radioactivity	No	Mouse	Oral	[redacted], USA	AD-120-2011
Tissue distribution of radioactivity	No	Rat	Oral	[redacted], USA	AD-120-2020

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption	No	Dog	Oral	[redacted], USA	AD-120-2009
Single dose tissue distribution	Yes	Dog	Oral	[redacted], USA	D990173-BP
Distribution into cerebrospinal fluid	No	Monkey	Oral	[redacted], USA	AD-120-2044 (8337908)
Studies in pregnant or nursing animals (FTC and TAF)					
Repeat-dose tissue distribution	No	Pregnant Mouse and Fetus	Oral	[redacted], USA	TOX103 Report Addendum
Repeat-dose tissue toxicokinetics on embryo/fetus	No	Rabbit	Oral	[redacted], USA	TOX038 Report Addendum
Single/repeat dose tissue distribution to evaluate placental transfer of TFV	No	Pregnant Rhesus Monkey and Fetus	Subcutaneous	[redacted], USA (In-life phase); Gilead Sciences, Foster City, CA, USA (Analysis)	96-DDM-1278-005
Single dose tissue distribution of TFV	No	Lactating Rhesus Monkey	Subcutaneous	[redacted], USA; [redacted] Canada (Analysis)	P2000116
Metabolism (BIC)					
Liver microsome stability	No	Liver Microsomes (Rat, Dog, Cynomolgus Monkey, Rhesus Monkey and Human)	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2289

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Metabolite identification	No	Cryopreserved Hepatocytes (Rat, Dog, Cynomolgus Monkey and Human)	In Vitro	[redacted]	AD-141-2288
Cytochrome P450 phenotyping	No	Human Liver Microsomes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2290
UDP-glucuronosyl transferase phenotyping	No	cDNA expressed human UGT enzyme preparations	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2291
Metabolite identification and profiling of radioactivity	No	Mouse	Oral	[redacted]	AD-141-2304 (8316891)
Metabolite identification and profiling of radioactivity	No	Rat	Oral	[redacted]	AD-141-2277 (8292820)
Metabolite identification and profiling of radioactivity	No	Cynomolgus Monkey	Oral	[redacted]	AD-141-2299 (8303909)
Metabolism (FTC)					
Metabolic reaction phenotyping	No	In Vitro	In Vitro	[redacted], Canada	15396V1 (48170, PDM-007)
Metabolism, excretion	Yes	Mouse	Oral	[redacted], USA	TEIN/93/0015
Metabolism, excretion	Yes	Cynomolgus Monkey	Oral	[redacted], USA	TEIN/93/0016
Metabolism, excretion	No	Cynomolgus Monkey	Oral	[redacted], USA	TOX063

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Metabolism (TAF, TFV)					
In vitro metabolism, plasma stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2025
In vitro metabolism, hepatic S9 stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2023
In vitro metabolism, intestinal S9 stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2024
In Vitro cytochrome P450 phenotyping	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2004
Metabolism in vitro	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2017
Metabolism in vitro of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	96-DDM-1278-003
Metabolite identification, mouse	No	Mouse	Oral	[redacted], USA	AD-120-2012
Metabolite identification, rat	No	Rat	Oral	[redacted], USA	AD-120-2021
Metabolite identification, dog	No	Dog	Oral	[redacted], USA	AD-120-2008
In vivo metabolism	No	Rhesus Monkey	Subcutaneous	[redacted]	P2001025
Excretion (BIC)					
Excretion of radioactivity	No	Mouse	Oral	[redacted]	AD-141-2303 (8316890)
Biliary excretion	No	BDC Rat	IV	[redacted]	AD-141-2283 (BG-0446-DA-RE)

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Excretion of radioactivity	No	Intact and BDC Monkeys	Oral	[redacted]	AD-141-2298 (8303908)
Excretion (TAF)					
Absorption and excretion	No	Dog	Oral	[redacted], USA	AD-120-2007
Absorption and excretion of TFV	No	Rat	IV	Gilead Sciences, Inc., Foster City, CA, USA	96-DDM-1278-001
Absorption and excretion of TFV	No	Dog	IV	[redacted], USA	96-DDM-1278-002
Pharmacokinetic Drug Interactions (BIC)					
Inhibition of human P-gp and BCRP	No	Transfected MDCKII Cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2273
OATP1B1 and OATP1B3 inhibition potential	No	Transfected CHO cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2274
OATP1B1 and OATP1B3 substrate	No	Transfected CHO cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2275
P-gp and BCRP substrate	No	Transfected MDCKII cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2278
Inhibition of OCT2 and MATE1	No	Transfected MDCKII Cells	In Vitro	Gilead Sciences, Inc., Foster City, CA, and [redacted], Hungary	AD-141-2285 ([redacted]-Gilead-64-05Sept2013)
Induction of metabolism enzymes	No	Reporter Cell-lines	In Vitro	[redacted]	AD-141-2292 (GIL-201212-132 and GIL-201213-141)
Cytochrome P450 inhibition potential	No	Human Liver Microsomes	In Vitro	[redacted], UK	AD-141-2293 (174-R276)

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
UGT1A1 inhibition potential	No	Human Liver Microsomes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-141-2294
Induction potential in human hepatocytes	No	Human Hepatocytes	In Vitro	[redacted]	AD-141-2305 (60N-1510)
Cytochrome P450 mechanism-based inhibition	No	Human Liver Microsomes	In Vitro	[redacted], UK	AD-141-2308 (174-R321)
Inhibition of OAT1, OAT3, OCT1 and BSEP	No	Transfected CHO, Flp-In 293 Cells or Sf9 Cell Membrane Vesicles	In Vitro	[redacted], Hungary	AD-141-2310 ([redacted]-Gilead-97-05Jun2015)
Drug-drug interaction liability assessment	No	NA	NA	NA	AD-141-2313
Pharmacokinetic Drug Interactions (FTC)					
Cytochrome P450 and UDP glucuronosyl transferase inhibition potential	No	In Vitro	In Vitro	[redacted], Canada	15247 (48171, PDM-006)
Induction potential of metabolizing enzymes	No	In Vitro	In Vitro	[redacted], USA	AD-162-2005
Pharmacokinetic Drug Interactions (TAF)					
In vitro cytochrome P450 inhibition	No	In Vitro	In Vitro	[redacted], UK	AD-120-2003
In vitro cytochrome P450 inhibition of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	V990172-104
In vitro cytochrome P450 mechanism-based inhibition	No	In Vitro	In Vitro	[redacted], UK	AD-120-2040
In Vitro cytochrome P450 induction	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2005

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Human UGT1A1 inhibition potential	No	In Vitro	In Vitro	[redacted], UK	AD-120-2006
Permeability in P-gp and BCRP overexpressing cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2018
Inhibition of human OATP1B1, OATP1B3, P-gp, and BCRP	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2019
Permeability across Caco-2 cell monolayers	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2013
Effect on uptake in human OATP1B1 and OATP1B3	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2022
Effect of pharmacoenhancer on intestinal stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2027
Inhibition of human OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP	No	In Vitro	In Vitro	[redacted], Hungary	AD-120-2036
Effect of cathepsin A, carboxylesterase 1, CYP3A4 on primary human hepatocytes	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2031
Human hepatocyte induction potential	No	In Vitro	In Vitro	[redacted], USA	AD-120-2032
Effect of TFV on human OAT3, OCT1, and OCT2	No	In Vitro	In Vitro	[redacted], USA; Gilead Sciences, Inc., Foster City, CA, USA	PC-103-2001
Transport of TFV by MRP2 and MRP4	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-104-2001
Assessment of interaction potential between TFV and human P-gp	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-104-2002
Drug interaction with human OAT1 transport of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-104-2010

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Drug interaction with human OAT3 transport of TFV	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-104-2011
Assessment of interaction potential between TFV and human MRP1	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	PC-104-2014
Effects of TFV on human OCT2 and MATE1	No	In Vitro	In Vitro	[redacted], USA; SOLVO Biotechnology, Budaörs, Hungary	AD-104-2012
Effect of CsA pretreatment on pharmacokinetics	No	Dog	IV, Oral	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2035
Pharmacokinetic Drug Interactions (EVG/COBI/FTC/TFV)					
Human OCT2 and MATE1 inhibition potential	No	In Vitro	In Vitro	[redacted], Hungary	AD-236-2001
Human P-gp and BCRP inhibition potential	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2003
Permeability in P-gp overexpressing cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2004
Permeability in BCRP overexpressing cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2005
Inhibition of human OATP1B1 and OATP1B3	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2006
Inhibition of human OAT1, OAT3, and MRP4 transporters	No	In Vitro	In Vitro	[redacted], Hungary	AD-236-2007
Inhibition of human OCT1 and BSEP transporters	No	In Vitro	In Vitro	[redacted], Hungary	AD-236-2008
Interaction of emtricitabine with human OAT1 and OAT3	No	In Vitro	In Vitro	[redacted], Hungary	AD-236-2010
Interaction of emtricitabine and tenofovir with human OCT1	No	In Vitro	In Vitro	[redacted], Hungary	AD-236-2011

Test Article: BIC, FTC, TAF, TFV, and/or TDF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Inhibition of elvitegravir and emtricitabine with human MRP2	No	In Vitro	In Vitro	[redacted], Hungary	AD-236-2012
Interaction of emtricitabine with human MRP2	No	In Vitro	In Vitro	[redacted], Hungary	AD-236-2013

Other Pharmacokinetic Studies: None

BCRP = breast cancer resistance protein; BDC = bile duct-cannulated; BIC = bictegravir (GS-9883); BSEP = bile salt export pump; Caco-2 = human colon carcinoma cell line; CHO = Chinese hamster ovary; CRO = contract research organization; CsA = cyclosporine A; CYP = cytochrome P 450 enzyme; Flp-In 293 = Parental cells transfected with pFRT/lacZeo and selected for stable Zeocin™ resistant clones; FTC = emtricitabine; Gilead = Gilead Sciences; GLP = Good Laboratory Practice; IV = intravenous; MATE = multidrug and toxin extrusion protein; MDCKII = Madin-Darby canine kidney cell line; MRP = multidrug resistance-associated protein; NA = not applicable; OAT = organic anion transporter; OATP = organic anion-transporting polypeptide; OCT = organic cation transporter; PBMC = peripheral blood mononuclear cell; P-gp = P-glycoprotein; Sf9 = Spodoptera frugiperda ovarian cells; TAF = tenofovir alafenamide; TDF = tenofovir disoproxil fumarate; TFV = tenofovir; UDP = uridine diphosphate; UGT = UDP glucuronosyl transferase

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

2. PHARMACOKINETICS: ANALYTICAL METHODS AND VALIDATION REPORTS

2.1. BIC

Test Article: BIC

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrix**	**Analyte**	**Analytical Method**
BA-141-2008[a]	Mouse Plasma	BIC	LC-MS/MS
BA-141-2001[a]	Rat Plasma	BIC	LC-MS/MS
BA-141-2007[b]	Rat Plasma	BIC	LC-MS/MS
BA-141-2006[b]	Rabbit Plasma	BIC	LC-MS/MS
BA-141-2002[a]	Monkey Plasma	BIC	LC-MS/MS

BIC = bictegravir (GS-9883); LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry
a Method validation
b Partial method validation

2.2. FTC

Test Article: FTC

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
97/001.01	Mouse, Monkey, Human Plasma and Human Urine	FTC	LC/MS (SIM)
6159v1	Mouse, Rabbit, Monkey Plasma	FTC	LC-MS/MS
6447v5	Mouse, Rat, Human Plasma	FTC	LC-MS/MS
7582v1	Monkey, Human Urine	FTC	LC-MS/MS

FTC = emtricitabine; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry

2.3. TAF

Test Articles: TAF, TFV

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
001092/NGE	Rat Plasma	TFV	LC-MS/MS
R-BA-Tox-120-001	Rat Plasma	TFV	LC-MS/MS
BA-120-2004	Mouse Plasma	TAF, TFV	LC-MS/MS
BA-120-2003	Rat Plasma	TAF, TFV	LC-MS/MS
BA-120-2005	Rabbit Plasma	TAF, TFV	LC-MS/MS
993680 MYS	Dog PBMC	TFV	LC-MS/MS
TOX-120-002, Appendix 32	Dog, Monkey, Human Plasma	TAF, TFV	LC-MS/MS
BA-120-2010	Monkey Plasma	TAF	LC-MS/MS
BA-120-2011	Monkey Plasma	TFV	LC-MS/MS
BA-120-2012	Monkey PBMC	TFV	LC-MS/MS
BA-120-2013	Monkey PBMC	TFV	LC-MS/MS
010520/PDW	Monkey Plasma	TFV	LC-MS/MS
010521/PHZ	Monkey Plasma	TAF	LC-MS/MS
AA01240-RQZ	Monkey PBMC	TFV	LC-MS/MS

LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir

2.4. TFV

Test Article: TFV

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
P4331-00008	Mouse Plasma	TFV	LC/Fluorescence
P1278-00001	Rat, Monkey Plasma	TFV	LC/Fluorescence
P1278-00028	Rat Plasma	TFV	LC-MS/MS
P1278-00034	Rat Milk	TFV	LC-MS/MS
P4331-035-3	Rabbit Plasma	TFV	LC/Fluorescence
P1278-00017	Dog Plasma	TFV	LC/Fluorescence
P4331-0037	Dog Plasma	TFV	LC-MS/MS
P1278-00029	Monkey Plasma	TFV	LC-MS/MS

LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; TFV = tenofovir

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

3.1. BIC

3.1.1. AD-141-2295: Membrane Permeability of BIC (In Vitro)

Report Title	Study Type	Test Article	Report Number
Bi-directional Permeability of GS-9883 through Caco-2 Cell Monolayers	Absorption Study (in vitro)	BIC	AD-141-2295

Study System	Caco-2 cell monolayers
Method	The bi-directional permeability of BIC through monolayers of Caco-2 cells in 24-well transwell plates was determined at 10 µM and 88 µM of BIC.

	P_{app} ($\times 10^{-6}$ cm/sec)		
Incubation Concentration (µM)	**Forward (A→B) Direction**	**Reverse (B→A) Direction**	**Efflux Ratio**
10	6.2	27.2	4.4
88	14.8	22.6	1.5

A = apical; B = basolateral; BIC = bictegravir (GS-9883); Caco-2 = human colon carcinoma cell line; P_{app} = apparent permeability coefficient
Experiment done with duplicate wells and values reported were the mean of two wells.
Assay control compounds: atenolol ($P_{app} < 1 \times 10^{-6}$ cm/s), propranolol ($P_{app} > 8 \times 10^{-6}$ cm/s), and vinblastine (efflux ratio > 20)

3.1.2. AD-141-2307: Pharmacokinetics of BIC in Mice

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of GS-9883 Following Single Ascending Oral Doses of GS-9883 to Male and Female Transgenic Mice	Single-Dose Pharmacokinetics	BIC	AD-141-2307

Species/Strain	Mouse/Transgenic rasH2 hemizygous, Model 1178-tg/wt [CByB6F1-Tg(HRAS)2Jic]									
Vehicle/Formulation	0.5% HPMC K100LV and 0.1% Tween 20 in water									
Method of Administration	Oral gavage									
Sample	Plasma									
Assay	LC/UV									
Analyte	BIC									
Salt Form	Sodium Salt									
Feeding Condition	Non-Fasted									
Sex/ No. of Animals	M/12	F/12	M/12	F/12	M/12	F/12	M/12	F/12	M/12	F/12
Dose (mg/kg)	30	30	100	100	300	300	1000	1000	1500	1500
PK Parameters										
T_{max} (h)	0.5	2.0	1.0	8.0	4.0	8.0	0.5	2.0	4.0	2.0
C_{max} (µg/mL)	59.3 ± 22.2	71.8 ± 7.3	97.9 ± 16.9	108 ± 16	116 ± 10	127 ± 15	135 ± 7	163 ± 13	123 ± 6	164 ± 2
$AUC_{0\text{-}24h}$ (µg•h/mL)	660 ± 34	745 ± 37	1257 ± 53	1509 ± 141	2106 ± 107	2173 ± 232	2568 ± 100	3197 ± 301	2155 ± 110	2366 ± 143
C_{24h} (µg/mL)	5.88 ± 0.90	5.66 ± 1.48	8.98 ± 1.89	11.1 ± 3.8	24.2 ± 6.2	20.2 ± 2.4	43.3 ± 19.8	57.9 ± 15.7	45.3 ± 28.3	37.4 ± 36.6

$AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from zero to 24 h; BIC = bictegravir (GS-9883); C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; F = female; HPMC = hydroxypropyl methylcellulose; LC/UV = high performance liquid chromatography coupled with UV detection; M = male; Non-Fasted = animals had access to Certified Rodent Diet *ad libitum*; PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration.

3.1.3. AD-141-2279: Pharmacokinetics of BIC in Rats

Report Title Single Dose Pharmacokinetic Study of GS-9883 in Male Sprague-Dawley Rats	**Study Type** Single-Dose Pharmacokinetics	**Test Article** BIC	**Report Number** AD-141-2279
Species/Strain	Rat/Sprague-Dawley		
Sex/ No. of Animals per group	Male / 3		
Feeding Condition	Fasted		
Vehicle/Formulation	5% ethanol, 55% PEG 300 and 40% water		
Sample	Plasma		
Dose (mg/kg)	0.5		
Analyte	BIC		
Salt Form	Free Acid		
Assay	LC-MS/MS		
Method of Administration	30 minute Intravenous Infusion	Oral gavage	
PK Parameters			
T_{max} (h)	0.58 ± 0.00	4.00 ± 2.00	
C_{max} (nM)	16100 ± 2350	3480 ± 773	
MRT (h)	45.7 ± 1.7	ND	
$AUC_{0\text{-}72h}$ (nM•h)	188000 ± 31700	107000 ± 38300	
AUC_{inf} (nM•h)	246000 ± 39400	125000 ± 43000	
$t_{1/2}$ (h)	32.4 ± 1.2	25.7 ± 1.9	
CL (L/h/kg)	0.0049 ± 0.0007	NA	
V_{ss} (L/kg)	0.22 ± 0.04	NA	
Bioavailability (%)	NA	49.8 ± 16.8	

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; BIC = bictegravir (GS-9883); CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; NA = not applicable; ND = not determined; PEG = polyethylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state
BIC: 1 nM = 0.000449 µg/mL

3.1.4. AD-141-2286: Pharmacokinetics of BIC in Rats

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of GS-9883 Following Single Ascending Oral Doses in Male Wistar Han Rats	Single-Dose Pharmacokinetics	BIC	AD-141-2286

Species/Strain	Rat/Wistar Han				
Sex/ No. of Animals per group	Male / 3				
Feeding Condition	Non-Fasted				
Vehicle/Formulation	0.5% HPMC K100LV and 0.1% Tween 20 in water				
Method of Administration	Oral gavage				
Sample	Plasma				
Analyte	BIC				
Salt Form	Free Acid				
Assay	LC/UV				
Dose (mg/kg)	10	30	100	300	1000[a]
PK Parameters					
T_{max} (h)	2.7 ± 1.2	2.3 ± 1.5	4.8 ± 3.9	10.7 ± 11.7	1.7 ± 0.6
C_{max} (µg/mL)	31.1 ± 6.0	54.3 ± 5.8	104 ± 30	120 ± 23	115 ± 14
AUC_{0-24h} (µg•h/mL)	471 ± 142	849 ± 66	1625 ± 826	2205 ± 248	1931 ± 109
C_{24h} (µg/mL)	14.5 ± 4.2	21.7 ± 3.2	46.2 ± 28.3	88.5 ± 18.4	41.8 ± 13.4

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; BIC = bictegravir (GS-9883); C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; HPMC = hydroxypropyl methylcellulose; LC/UV = high performance liquid chromatography coupled with UV detection; Non-Fasted = animals had access to Certified Rodent Diet *ad libitum;* PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration

a Formulation contained 0.5% HPMC K100LV, 0.1% Tween 20 and 0.9% benzyl alcohol in water.

3.1.5. AD-141-2296: Pharmacokinetics of BIC in Rats

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of GS-9883 (Sodium Salt) Following Single Ascending Oral Doses in Male Wistar Han Rats	Single-Dose Pharmacokinetics	BIC	AD-141-2296
Species/Strain	Rat/Wistar Han		
Sex/ No. of Animals per group	Male / 3		
Feeding Condition	Non-Fasted		
Vehicle/Formulation	0.5% HPMC and 0.1% Tween 20 in water		
Method of Administration	Oral gavage		
Sample	Plasma		
Analyte	BIC		
Salt Form	Sodium Salt		
Assay	LC/UV		
Dose (mg/kg)	30	100	300
PK Parameters			
T_{max} (h)	3.67 ± 3.79	5.00 ± 3.61	6.00 ± 2.00
C_{max} (µg/mL)	55.3 ± 6.0	102 ± 17	129 ± 9
AUC_{0-24h} (µg•h/mL)	926 ± 209	1896 ± 331	2436 ± 481
C_{24h} (µg/mL)	27.3 ± 5.4	71.0 ± 26.4	71.5 ± 31.0

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; BIC = bictegravir (GS-9883); C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; HPMC = hydroxypropyl methylcellulose; LC/UV = high performance liquid chromatography coupled with UV detection; Non-Fasted = animals had access to Certified Rodent Diet *ad libitum;* PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration.

3.1.6. AD-141-2306: Pharmacokinetics of BIC in Rats

Report Title	**Study Type**	**Test Article**	**Report Number**	
Pharmacokinetics of GS-9883 Following Single Ascending Oral Doses of GS-9883 in solution to Male Wistar Han Rats	Single-Dose Pharmacokinetics	BIC	AD-141-2306	
Species/Strain	Rat/Wistar Han			
Sex/ No. of Animals per group	Male / 3			
Feeding Condition	Non-Fasted			
Vehicle/Formulation	10% ethanol, 10% propylene glycol, 40% Labrasol and 40% Solutol® HS 15			
Method of Administration	Oral gavage			
Sample	Plasma			
Analyte	BIC			
Salt Form	Free Acid			
Assay	LC/UV			
Dose (mg/kg)	10	30	100	300
PK Parameters				
T_{max} (h)	6.0 ± 2.0	2.7 ± 1.2	2.3 ± 1.5	13.3 ± 10.1
C_{max} (µg/mL)	61.5 ± 2.1	114 ± 5	148 ± 7	105 ± 26
$AUC_{0\text{-}24h}$ (µg•h/mL)	929 ± 97	1904 ± 249	2847 ± 229	2137 ± 570
C_{24h} (µg/mL)	23.8 ± 4.0	49.8 ± 9.6	84.4 ± 14.1	79.5 ± 8.9

$AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from zero to 24 h; BIC = bictegravir (GS-9883); C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; LC/UV = high performance liquid chromatography coupled with UV detection; Non-Fasted = animals had access to Certified Rodent Diet *ad libitum*; PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration.

3.1.7. AD-141-2300: Pharmacokinetics of BIC in Rabbits

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of GS-9883 Following Single Ascending Oral Doses of GS-9883 to Female New Zealand White Rabbits	Single-Dose Pharmacokinetics	BIC	AD-141-2300

Species/Strain	Rabbit/New Zealand White		
Sex/ No. of Animals per group	Female / 3		
Feeding Condition	Non-Fasted		
Vehicle/Formulation	0.5% HPMC K100LV and 0.5% Tween 20 in water		
Method of Administration	Oral gavage		
Sample	Plasma		
Analyte	BIC		
Salt Form	Sodium Salt		
Assay	LC-MS/MS		
Dose (mg/kg)	100	300	1000
PK Parameters			
T_{max} (h)	1.67 ± 0.58	2.00 ± 0.00	16.7 ± 12.7
C_{max} (µg/mL)	4.38 ± 0.21	6.41 ± 1.73	9.76 ± 3.49
$AUC_{0\text{-}24h}$ (µg•h/mL)	23.3 ± 1.9	69.7 ± 6.9	171 ± 64
C_{24h} (µg/mL)	0.32 ± 0.12	1.82 ± 0.37	9.29 ± 4.30

$AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from zero to 24 h; BIC = bictegravir (GS-9883); C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; HPMC = hydroxypropyl methylcellulose; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration.

3.1.8. AD-141-2280: Pharmacokinetics of BIC in Dogs

Report Title Single Dose Pharmacokinetic Study of GS-9883 in Male Beagle Dogs	**Study Type** Single-Dose Pharmacokinetics	**Test Article** BIC	**Report Number** AD-141-2280
Species/Strain	Dog /Beagle		
Sex/ No. of Animals per group	Male / 3		
Feeding Condition	Fasted		
Sample	Plasma		
Analyte	BIC		
Salt Form	Free Acid		
Assay	LC-MS/MS		
Vehicle/Formulation	5% ethanol, 55% PEG 300, and 40% water	30% Captisol and 70% water	
Method of Administration	30 minute Intravenous Infusion	Oral gavage	
Dose (mg/kg)	0.5	1.0	
PK Parameters			
T_{max} (h)	0.55 ± 0.06	0.83 ± 0.29	
C_{max} (nM)	8600 ± 327	9720 ± 1130	
MRT (h)	7.10 ± 1.32	ND	
AUC_{0-24h} (nM•h)	55900 ± 16100	54800 ± 17600	
AUC_{inf} (nM•h)	58700 ± 17700	55900 ± 18500	
$t_{1/2}$ (h)	5.34 ± 0.18	4.26 ± 0.40	
CL (L/hr/kg)	0.022 ± 0.006	NA	
V_{ss} (L/kg)	0.15 ± 0.02	NA	
Bioavailability (%)	NA	41.8 ± 13.9	

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; BIC = bictegravir (GS-9883); CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; NA = not applicable; ND = not determined; PEG = polyethylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state
BIC: 1 nM = 0.000449 µg/mL

3.1.9. AD-141-2281: Pharmacokinetics of BIC in Cynomolgus Monkeys

Report Title Single Dose Pharmacokinetic Study of GS-9883 in Male Cynomolgus Monkeys	**Study Type** Single-Dose Pharmacokinetics	**Test Article** BIC	**Report Number** AD-141-2281
Species/Strain	Monkey/Cynomolgus		
Sex/ No. of Animals per group	Male / 3		
Feeding Condition	Fasted		
Sample	Plasma		
Analyte	BIC		
Salt Form	Free Acid		
Assay	LC-MS/MS		
Vehicle/Formulation	5% ethanol, 55% PEG 300, and 40% water	30% Captisol and 70% water	
Method of Administration	30 minute Intravenous Infusion	Oral gavage	
Dose (mg/kg)	0.5	1.0	
PK Parameters			
T_{max} (h)	0.55 ± 0.06	0.83 ± 0.29	
C_{max} (nM)	11500 ± 173	16600 ± 4540	
MRT (h)	4.16 ± 0.93	ND	
AUC_{0-24h} (nM•h)	49000 ± 12200	72100 ± 39300	
AUC_{inf} (nM•h)	49400 ± 12400	72500 ± 39500	
$t_{1/2}$ (h)	3.58 ± 0.23	3.26 ± 0.50	
CL (L/h/kg)	0.024 ± 0.007	NA	
V_{ss} (L/kg)	0.095 ± 0.010	NA	
Bioavailability (%)	NA	73.8 ± 40.3	

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; BIC = bictegravir (GS-9883); CL = plasma clearance; C_{max} = maximum plasma concentration; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; NA = not applicable; ND = not determined; PEG = polyethylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state
Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing.
BIC: 1 nM = 0.000449 µg/mL

3.1.10. AD-141-2284: Pharmacokinetics of BIC in Cynomolgus Monkeys

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of GS-9883 Following Single Ascending Oral Doses in Male Cynomolgus Monkeys	Single-Dose Pharmacokinetics	BIC	AD-141-2284

Species/Strain	Cynomolgus Monkeys				
Sex/ No. of Animals per group	Male / 3				
Feeding Condition	Non-Fasted				
Vehicle/Formulation	0.5% HPMC K100LV, 0.1% Tween 20, and 0.9% benzyl alcohol in water				
Method of Administration	Oral gavage				
Sample	Plasma				
Analyte	BIC				
Salt Form	Free Acid				
Assay	LC/UV				
Dose (mg/kg)	10	30	100	300	1000
PK Parameters					
T_{max} (h)	3.33 ± 1.15	4.00 ± 0.00	5.33 ± 1.15	4.67 ± 1.15	6.67 ± 2.31
C_{max} (µg/mL)	10.3 ± 2.0	18.9 ± 1.3	47.1 ± 10.8	63.8 ± 6.7	82.6 ± 9.9
AUC_{0-24h} (µg•h/mL)	95.6 ± 25.6	197 ± 14	584 ± 133	804 ± 220	1078 ± 166
C_{24h} (µg/mL)	0.88 ± 0.46	2.08 ± 0.87	5.39 ± 2.95	14.0 ± 11.1	16.7 ± 8.8

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; BIC = bictegravir (GS-9883); C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; HPMC = hydroxypropyl methylcellulose; LC/UV = high performance liquid chromatography coupled with UV detection; PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration
Non-Fasted = animals had access to Certified Primate Diet #2055C (Harlan)

3.1.11. AD-141-2297: Pharmacokinetics of BIC in Cynomolgus Monkeys

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of GS-9883 (Sodium Salt) Following Single Ascending Oral Doses in Male Cynomolgus Monkeys	Single-Dose Pharmacokinetics	BIC	AD-141-2297

Species/Strain	Cynomolgus Monkeys		
Sex/ No. of Animals per group	Male / 3		
Feeding Condition	Non-Fasted		
Vehicle/Formulation	0.5% HPMC and 0.1% Tween 20 in water		
Method of Administration	Oral gavage		
Sample	Plasma		
Analyte	BIC		
Salt Form	Sodium Salt		
Assay	LC/UV		
Dose (mg/kg)	30	100	1000
PK Parameters			
T_{max} (h)	2.67 ± 1.15	2.67 ± 1.15	5.33 ± 1.15
C_{max} (µg/mL)	18.7 ± 4.0	42.1 ± 10.3	80.9 ± 25.6
AUC_{0-24h} (µg•h/mL)	171 ± 73	348 ± 51	1056 ± 339
C_{24h} (µg/mL)	2.32 ± 1.93	3.42 ± 1.21	13.4 ± 3.6

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; BIC = bictegravir (GS-9883); C_{max} = maximum plasma concentration; C_{24h} = measured concentration at 24 h post dose; HPMC = hydroxypropyl methylcellulose; LC/UV = high performance liquid chromatography coupled with UV detection; PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration
Non-Fasted = animals had access to Certified Primate Diet #2055C (Harlan)

3.1.12. AD-141-2282: Pharmacokinetics of BIC in Rhesus Monkeys

Report Title Single Dose Pharmacokinetic Study of GS-9883 in Male Rhesus Monkeys	**Study Type** Single-Dose Pharmacokinetics	**Test Article** BIC	**Report Number** AD-141-2282
Species/Strain	Monkey/Rhesus		
Sex/ No. of Animals	Male / 3		
Feeding Condition	Fasted		
Vehicle/Formulation	5% ethanol, 55% PEG 300, and 40% water		
Method of Administration	30 minute Intravenous Infusion		
Sample	Plasma		
Dose (mg/kg)	0.5		
Analyte	BIC		
Salt Form	Free Acid		
Assay	LC-MS/MS		
PK Parameters			
T_{max} (h)	0.58 ± 0.00		
C_{max} (nM)	9450 ± 906		
MRT (h)	4.36 ± 1.30		
AUC_{0-24h} (nM•h)	42500 ± 4610		
AUC_{inf} (nM•h)	43000 ± 5050		
$t_{1/2}$ (h)	3.76 ± 0.76		
CL (L/h/kg)	0.026 ± 0.003		
V_{ss} (L/kg)	0.11 ± 0.02		

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; BIC = bictegravir (GS-9883); CL = plasma clearance; C_{max} = maximum plasma concentration; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; PEG = polyethylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state
Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing
BIC: 1 nM = 0.000449 µg/mL

3.2. FTC

3.2.1. TEIN/93/0003: Pharmacokinetics of FTC in Mice (10 mg/kg)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of 524W91 in Male CD-1 Mice Following Oral and Intravenous Administration	Absorption, bioavailability	FTC	TEIN/93/0003

Species	Mouse / CD-1
Feeding Condition	Not fasted
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous bolus
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection
Dose (mg/kg)	10 oral/ 10 IV
Sex (M/F)/Number of Animals	120 M / dose

PK Parameters	Oral	IV
T_{max} (min)	25.4	-
C_{max} (µM)	9.8	-
AUC_{inf} (µM•h)	16.7	17.4
CL (L/h/kg)	-	2.33
$t_{1/2}\beta$ (min)	-	23
V_{ss} (L/kg)	-	0.89

Additional Information: Absolute bioavailability = 96%

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; FTC = emtricitabine; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

3.2.2. TEIN/93/0004: Pharmacokinetics of FTC in Mice (100 mg/kg)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of 100 mg/kg or Oral and Intravenous 524W91 in Male CD-1 Mice	Absorption, bioavailability	FTC	TEIN/93/0004

Species	Mouse / CD-1
Feeding Condition	Not fasted
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous bolus
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection
Dose (mg/kg)	100 oral/ 100 IV
Sex (M/F)/Number of Animals	120 M / dose

PK Parameters	Oral	IV
T_{max} (min)	24.5	-
C_{max} (μM))	89	-
AUC_{inf} (μM•h)	143	181
CL (L/h/kg)	-	2.23
$t_{1/2}$ β (min)	-	15.5
$t_{1/2}$ γ (min)	-	82
V_{ss} (L/kg)	-	0.94
Additional Information: Absolute bioavailability = 79%		

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; FTC = emtricitabine; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

3.2.3. IUW00101: Pharmacokinetics of FTC in Mice (600 mg/kg)

Report Title	Study Type	Test Article	Report Number
Pharmacokinetic Study in Male Mice Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine	Absorption, bioavailability	FTC	IUW00101

Species	Mouse / CD-1
Sex (M/F)/Number of Animals	Oral – 36 (numbered 4-39, 1-3 were bled predose) (male); IV – 36 (numbered 43-78, 40-42 were bled predose) (male)
Feeding Condition	Not fasted
Vehicle/Formulation	Oral – 60 mg/mL suspension with 0.5% hydroxypropylmethyl cellulose; IV – 60 mg/mL in phosphate buffered saline using distilled water, pH 7.2
Method of Administration	Oral and IV bolus
Dose (mg/kg)	600
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection

PK Parameters	Oral	IV
T_{max} (h)	0.667	0
C_{max} (µg/mL)	139	1560
$AUC_{0\text{-}last}$ (µg•h/mL)	270	465
AUC_{inf} (µg•h/mL)	296	473
CL (L/h/kg)	-	1.28
$t_{1/2}$ lambda (h)	3.17	4.14
λ_z (h^{-1})	0.219 (apparent terminal rate constant)	0.167 (apparent terminal rate constant)
V_{ss} (L/kg)	-	1.10
MAT (h)	2.04 (calculated as MRT_{po}-MRT_{IV})	-
MRT (h)	2.90 (calculated from AUMC/ AUC_{inf})	0.860 (calculated from AUMC/ AUC_{inf})
F	62.7%	-

λ_z = terminal elimination rate constant; $AUC_{0\text{-}last}$ = area under the plasma concentration-time curve from zero to last measured time-point; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; F = bioavailability; FTC = emtricitabine; IV = intravenous; MAT = mean absorption time; MRT = mean residence time; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

3.2.4. TEZZ/93/0019: Pharmacokinetics of FTC in Fasted Cynomolgus Monkeys

Report Title	**Study Type**	**Test Article**	**Report Number**
A Pharmacokinetic Study of 524W91 in Cynomolgus Monkeys Following Oral and Intravenous Administration	Absorption, bioavailability	FTC	TEZZ/93/0019

Species	Monkey / Cynomolgus
Sex (M/F)/Number of Animals	4 M / dose
Feeding Condition	Fasted
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous (crossover)
Dose (mg/kg)	10 and 80
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection

PK Parameters

Oral	**10 mg/kg**	**80 mg/kg**
C_{max} (μM)	14.1 ± 2.00	111 ± 34.0
T_{max} (h)	1.3 ± 0.50	2.30 ± 0.29
AUC_{inf} (μM•h)	37.4 ± 6.5	285 ± 82.6
CL/F (L/h/kg)	1.1 ± 0.19	1.2 ± 0.31
Intravenous	**10 mg/kg**	**80 mg/kg**
AUC_{inf} (μM•h)	59.9 ± 11.4	493 ± 65.4
CL (L/h/kg)	0.70 ± 0.14	0.70 ± 0.08
V_{ss} (L/kg)	0.80 ± 0.02	0.80 ± 0.09
$t_{1/2}\beta$ (h)	1.00 ± 0.19	1.02 ± 0.13

Additional Information: Absolute bioavailability 44 to 69%.

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; F = bioavailability; FTC = emtricitabine; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

3.2.5. IUW00301: Pharmacokinetics of FTC in Nonfasted Cynomolgus Monkeys

Report Title	Study Type	Test Article	Report Number
Pharmacokinetic Study in Male cynomolgus Monkeys Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine	Absorption, bioavailability	FTC	IUW00301

Species	Monkey / Cynomolgus
Sex (M/F)/Number of Animals	4 M / dose
Feeding Condition	Nonfasted
Vehicle/Formulation	Oral: 32.0 mg/mL aqueous solution, Intravenous: 81.8 mg/mL in phosphate buffered saline, pH 7.2
Method of Administration	Oral and intravenous (crossover)
Dose (mg/kg)	80
Sample	Plasma
Analyte	FTC
Assay	Validated HPLC with UV detection

Oral		Intravenous	
Plasma	Mean ± Standard Deviation	**Plasma**	Mean ± Standard Deviation
C_{max} (µg/mL)	39.4 ± 4.87	**C_{max} (µg/mL)**	238 ± 46.0 (end of infusion)
T_{max} (h)	0.884 (median)	**T_{max} (h)**	Not applicable
$AUC_{0\text{-}last}$ (µg•h/mL)	83.0 ± 11.1	**$AUC_{0\text{-}last}$ (µg•h/mL)**	85.5 ± 17.3
AUC_{inf} (µg•h/mL)	83.6 ± 11.2	**AUC_{inf} (µg•h/mL)**	86.1 ± 17.3
CL/F (L/h/kg)	Not calculated	**CL/F (L/h/kg)**	0.970 ± 0.158
$t_{1/2}$ lambda (h)	0.936 ± 0.0909	**$t_{1/2}$ lambda (h)**	0.775 ± 0.0586
λ_z (h^{-1})	0.746 ± 0.0793	**λ_z (h^{-1})**	0.898 ± 0.0640
MAT (h)	0.953 ± 0.154 (calculated as MRT_{po} - MRT_{iv})	**MRT (h)**	0.802 ± 0.0793 (calculated from $AUMC/AUC_{inf}$)
MRT (h)	1.75 ± 0.156 (calculated from $AUMC/AUC_{inf}$)	**V_{ss} (L/kg)**	0.769 ± 0.0743
F (%)	97.4 ± 6.98		

λ_z = terminal elimination rate constant; $AUC_{0\text{-}last}$ = area under the plasma concentration-time curve from zero to last measured time-point; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; F = bioavailability; FTC = emtricitabine; MAT = mean absorption time; MRT = mean residence time; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration

3.3. TAF

3.3.1. AD-120-2037: Caco-2 Permeability of TAF (In Vitro)

Report Title:	**Study Type**	**Test Article**	**Report Number**
Concentration Dependent Permeability of Tenofovir Alafenamide through Caco-2 Cell Monolayers	Absorption (in vitro)	TAF	AD-120-2037

Bidirectional Permeability of TAF Through Caco-2 Cells

Inhibitor	Direction	Target Concentration (µM)	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s) Average	Efflux Ratio
—	Cell-Free	10	11.3	106	42.9	20.2
	Forward		10.9	42.7	0.34	
	Reverse		10.0	85.3	6.98	
—	Cell-Free	100	104	112	53.2	13.6
	Forward		92.0	40.4	0.63	
	Reverse		98.1	85.2	8.47	
—	Cell-Free	1000	969	109	46.1	6.28
	Forward		933	77.4	1.08	
	Reverse		1038	88.8	5.86	
CsA	Cell-Free	10	9.85	113	43.5	1.00
	Forward		10.4	42.2	1.51	
	Reverse		10.8	70.5	1.34	

Caco-2 = human colonic adenocarcinoma cell line; TAF = tenofovir alafenamide; P_{app} = apparent permeability; CsA = cyclosporine A

3.3.2. AD-120-2014: Pharmacokinetics of TAF in Mice

Report Title:	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Mice	Absorption	TAF	AD-120-2014

Species: CD-1 mice

Feeding Condition: Not fasted

Vehicle/Formulation: 0.1% (v/v) tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in reverse osmosis water

Method of Administration: Oral gavage

Sample: Plasma

Assay: LC-MS/MS

Test Article	GS-7340-02						GS-7340-03					
Sex (M/F) / N of Animals	M/36						M/36					
Dose (mg/kg)	10		30		100		10		30		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
T_{max} (h)	0.08	0.50	NA	0.25	0.08	0.75	NA	0.50	4.00	0.50	0.25	1.50
C_{max} (ng/mL)	5.53	106	NA	440	37.1	1827	NA	85.4	10.3	383	34.7	2152
$t_{1/2}$ (h)	NA	NA	NA	NA	NA	NA	NA	5.16	NA	10.1	NA	NA
AUC_{0-t} (ng•h/mL)	NA	455	NA	2005	26.0	10643	NA	493	NA	2477	11.3	10866
T_{last} (h)	NA	12.0	NA	24.0	1.50	24.0	NA	12.0	NA	24.0	0.50	24.0
C_{last} (ng/mL)	NA	21.6	NA	35.9	12.8	157	NA	20.5	NA	34.4	24.9	205

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.3. AD-120-2016: Pharmacokinetics of TAF in Mice

Report Title:	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-03 After a Single Oral Gavage Dose to Male and Female 001178-W (wild type) Mice	Absorption	TAF	AD-120-2016

Species: Model 001178-W [Wild, CByB6F1-Tg(HRAS)2Jic] mice

Feeding Condition: Not fasted

Vehicle/Formulation: 0.1:0.1:99.8 (w/w/w) hydroxypropyl methylcellulose K100LV (HPMC)/polysorbate (tween) 20/reverse osmosis water

Method of Administration: Oral gavage

Sample: Plasma

Assay: LC-MS/MS

Test Article	**GS-7340-03**											
Sex (M/F) / N of Animals	**M/44**		**F/44**		**M/44**		**F/44**		**M/44**		**F/44**	
Dose (mg/kg)	**10**				**30**				**100**			
Analyte	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**
PK Parameters												
T_{max} (h)	NA	0.25	NA	0.50	0.08	0.25	0.50	0.25	0.25	0.50	0.50	0.50
C_{max} (ng/mL)	NA	175	NA	100	8.80	615	117	421	648	1988	280	1733
$t_{½}$ (h)	NA	9.78	NA	8.20	NA	9.51	NA	10.9	NA	8.04	NA	11.0
AUC_{0-t} (ng•h/mL)	NA	735	NA	354	NA	2639	NA	2053	194	10026	104	7131
T_{last} (h)	NA	24.0	NA	12.0	0.25	24.0	NA	24.0	0.50	24.0	0.50	24.0
C_{last} (ng/mL)	NA	13.5	NA	16.5	5.40	31.1	NA	36.3	61.4	99.9	280	113

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.4. AD-120-2015: Pharmacokinetics of TAF in Rats

Report Title:	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Rats	Absorption	TAF	AD-120-2015

Species: SD rats

Feeding Condition: Not fasted

Vehicle/Formulation: 0.1% (v/v) tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in reverse osmosis water

Method of Administration: Oral gavage

Sample: Plasma

Assay: LC-MS/MS

Test Article	GS-7340-02						GS-7340-03					
Sex (M/F) / N of Animals	M/3						M/3					
Dose (mg/kg)	5		25		100		5		25		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
T_{max} (h)	NA	0.67	NA	0.58	NA	0.83	NA	0.58	NA	0.83	NA	0.67
C_{max} (ng/mL)	NA	32.5	NA	199	NA	1240	NA	39.3	NA	364	NA	1670
$t_{½}$ (h)	NA	NA	NA	11.2	NA	10.3	NA	NA	NA	7.89	NA	7.85
AUC_{0-t} (ng•h/mL)	NA	122	NA	1395	NA	7771	NA	88.5	NA	1810	NA	9759
T_{last} (h)	NA	8.00	NA	24.0	NA	24.0	NA	4.67	NA	24.0	NA	24.0
C_{last} (ng/mL)	NA	10.5	NA	25.1	NA	156	NA	12.6	NA	19.4	NA	113

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.5. R990130: Pharmacokinetics of TAF in Rats

Report Title:	Study Type	Test Article	Report Number
Tenofovir (GS-1278) Plasma Pharmacokinetics Following a Single Oral Dose of GS-7340-02 in the Male Albino Rat	Absorption	TAF	R990130

Species:	Sprague-Dawley rats
Feeding Condition:	Fasted
Vehicle/Formulation:	50 mM citric acid
Method of Administration:	Oral gavage
Sample:	Plasma
Assay:	LC/Fluorescence

Test Article	**GS-7340-02**			
Sex (M/F) / N of Animals	**M/4**			
Dose (mg/kg)	**6.25**	**25**	**100**	**400**
Analyte	**TFV**			
PK Parameters				
T_{max} (h)	0.26	0.50	0.50	0.25
C_{max} (ng/mL)	104	1220	3870	15800
$t_{½}$ (h)	NA	14.5	16.2	17.3
AUC_{0-t} (ng•h/mL)	160	3270	12200	48300
T_{last} (h)	4.00	20.0	20.0	24.0
$MRT_{0-\infty}$ (h)	NA	20.8	18.9	20.6

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; C_{max} = maximum plasma concentration; F = female; M = male; MRT = mean residence time; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.6. R2000065: Pharmacokinetics of TAF in Rats

Report Title:	Study Type	Test Article	Report Number
Comparison of Plasma Pharmacokinetics in Rats of Tenofovir Following Oral Administration of GS-7340-02 or Tenofovir DF as Either a suspension in CMC or a Solution in Citric Acid	Absorption	TAF	R2000065

Species: Sprague-Dawley rats

Feeding Condition: Fasted

Dose (mg/kg): 400

Method of Administration: Oral gavage

Sample: Plasma

Assay: LC-MS/MS

Test Article	**GS-7340-02**		**TDF**	
Sex (M/F) / N of Animals	**M/3**			
Vehicle/Formulation	**CMC Suspension**	**50 mM Citric Acid**	**CMC Suspension**	**50 mM Citric Acid**
Analyte	**TFV**			
PK Parameters				
T_{max} (h)	0.50	0.25	0.25	0.50
C_{max} (ng/mL)	14229	8418	8101	2699
$t_{½}$ (h)	11.3	11.4	7.21	8.31
AUC_{0-t} (ng•h/mL)	36288	33067	15774	11403
AUC_{inf} (ng•h/mL)	36795	33638	15848	11581
T_{last} (h)	55.0	55.0	55.0	48.0

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; CMC = carboxymethylcellulose; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.7. 99-DDM-1278-001-PK: Pharmacokinetics of TAF in Dogs

Report Title:	Study Type	Test Article	Report Number
Analysis of Data from [redacted] Bioavailability Study M059-98 of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species:	Beagle dogs
Feeding Condition:	Fasted
Vehicle/Formulation:	Sterile Saline
Method of Administration:	IV bolus
Assay:	LC/Fluorescence

Test Article	**GS-7340-2**		
Sex (M/F) / N of Animals	**M/5**		
Dose (mg/kg)	**6.20**		
Sample	**Plasma**		**PBMC**
Analyte	**TAF**	**TFV**	**TFV**
PK Parameters			
T_{max} (h)	0.00*	0.04	7.6
C_{max} (µg/mL)	8.88*	2.09	26.2
$t_{½}$ (h)	0.13	13.7	> 24
AUC_{0-t} (µg•h/mL)	1.46	2.01	448
AUC_{inf} (µg•h/mL)	1.52	2.69	NC
V_z (L/kg)	0.82	NA	NA
CL (L/h/kg)	4.48	NA	NA

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; CL = plasma clearance; F = female; IV = intravenous; M = male; NA = not applicable; NC = not calculated; PBMC = peripheral blood mononuclear cells; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate; V_z = apparent volume of distribution during the terminal phase
* Extrapolated to time zero

3.3.8. AD-120-2034: Pharmacokinetics of TAF in Dogs

Report Title:	Study Type	Test Article	Report Number
Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (TAF) Following Single Oral Administration in Male Beagle Dogs	Absorption	TAF	AD-120-2034

Species:	Beagle dogs
Feeding Condition:	Fasted
Vehicle/Formulation:	0.1% (w/w) hydroxypropylmethylcellulose K100LV (HPMC) K100LV, 0.1% polysorbate 20 in water
Method of Administration:	Oral gavage[a]
Dose:	10 mg/kg
Assay:	LC-MS/MS

Test Article	**GS-7340-02**				
Sex (M/F) / N of Animals	**M/6**				
Sample	**Plasma**		**Liver**		
Analyte	**TAF**	**TFV**	**TFV**	**TFV-MP**	**TFV-DP**
PK Parameters					
T_{max} (h)	0.08	1.00	1.00	4.00	4.00
C_{max} (µg/mL)	3.49	0.64	12.7	12.6	56.4
$t_{½}$ (h)	0.24	> 24	15.9	> 24	22.3
AUC_{0-t} (µg•h/mL)	1.38	2.72	86.8	258	1017

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate
a Animals were pretreated with pentagastrin 20 minutes prior to the dose administration.

3.3.9. P2000087: Pharmacokinetics of TAF in Rhesus Monkeys

Report Title:	Study Type	Test Article	Report Number
A Single Dose Pharmacokinetic and Oral Bioavailability Study of GS-7340-02 in Rhesus Monkeys	Absorption, Bioavailability	TAF	P2000087

Species: Rhesus monkeys

Feeding Condition: Fasted

Vehicle/Formulation: 50 mM citric acid

Method of Administration: Nasogastric gavage

Sample: Plasma

Assay: LC-MS/MS

Test Article	GS-7340-02											
Dose (mg/kg)	0.5				5				50			
Sex (M/F) / N of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
T_{max} (h)	0.40	1.00	0.33	1.00	0.80	1.33	0.80	1.30	0.40	1.00	0.70	1.00
C_{max} (ng/mL)	3.92	8.50	1.65	6.94	161	169	88.6	152	5388	1576	2897	1076
$t_{½}$ (h)	NC	4.24	0.61	7.18	0.27	10.0	0.19	12.6	0.22	23.9	0.57	14.8
AUC_{0-t} (ng•h/mL)	1.85	34.0	0.60	45.9	131	1151	59.5	924	5038	13119	2583	6749
AUC_{inf} (ng•h/mL)	NC	43.5	2.47	62.0	77.3	1186	82.0	951	5109	13585	2584	6914
MRT (h)	0.43	6.72	0.59	11.0	0.98	11.8	0.88	11.8	0.77	20.0	0.90	15.9

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity ; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; MRT = mean residence time; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

3.3.10. P2000031: Pharmacokinetics of TDF in Rhesus Monkeys

Report Title:	Study Type	Test Article	Report Number
A Single Dose Oral Bioavailability Study of TDF in Rhesus Monkeys	Absorption, Bioavailability	TDF	P2000031

Species: Monkey
Feeding Condition: Fed & Fasted (oral)
Vehicle/Formulation: TDF (Citric acid)
Method of Administration: TDF (oral)
Sample: Plasma
Analyte: TFV
Assay: LC-MS/MS

Dose (mg/kg) (TDF)	**5 (Oral)**		**50 (Oral)**		**250 (Oral)**	
Sex (M/F) No. of Animals	**6 (3M, 3F) Fed**		**6 (3M, 3F) Fed**		**12 (6M, 6F) Fed + Fasted**	
PK Parameters (Oral) Mean (SD)						
C_{max} (µg/mL)	0.113	(0.042)	1.15	(0.676)	1.68	(1.05)
T_{max} (h)	0.83	(0.408)	1.00	(0.548)	1.08	(0.56)
AUC_{inf} (µg•h/mL)	0.725	(0.125)	6.38	(1.74)	14.8	(7.81)
AUC % Extrapolated	2.82	(0.768)	2.26	(1.00)	2.19	(0.710)
$t_{1/2}$ (h)	8.23	(1.06)	8.54	(1.14)	8.41	(1.20)
CL/F (mL/h/kg)	3202	(566)	3807	(1191)	6569	(2996)
C_{last} (µg/mL)	0.00169	(0.00027)	0.0118	(0.00615)	0.0366	(0.0102)
T_{last} (h)	44.0	(6.20)	48.0	(0.0)	48.0	(0.0)
V_z/F (mL/kg)	38000	(8538)	47250	(18111)	82600	(50113)
F (%)	32.4	(7.90)	23.7	(7.82)	17.0	(5.66)

AUC = area under the plasma concentration-time; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; CL = plasma clearance; F = female; F (%) = bioavailability; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; PK = pharmacokinetic; SD = standard deviation; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TDF = tenofovir disoproxil fumarate; TFV = tenofovir; V_z = apparent volume of distribution during the terminal phase

Report Title:	**Study Type**	**Test Article**	**Report Number**
A Single Dose Oral Bioavailability Study of TDF in Rhesus Monkeys	Absorption, Bioavailability	TDF	P2000031

Species:	Monkey
Feeding Condition:	Fed
Vehicle/Formulation:	TFV (physiological buffered solution)
Method of Administration:	TFV (IV)
Sample:	Plasma
Analyte:	TFV
Assay:	LC-MS/MS

Dose (mg/kg) (TFV)	**5 (IV)**		**30 (IV)**	
Sex (M/F) No. of Animals	**6 (3M/3F)**		**6 (3M/3F)**	
Tabulated PK for IV Dose Results Mean (SD)				
C_{max} (µg/mL)	13.8	(3.08)	79.0	(12.6)
AUC_{inf} (µg•h/mL)	5.12	(1.15)	38.4	(16.2)
AUC % Extrapolated	0.520	(0.394)	0.199	(0.0841)
$t_{1/2}$ (h)	5.37	(1.35)	8.79	(2.79)
CL (mL/h/kg)	1031	(301)	888	(315)
C_{last} (µg/mL)	0.00419	(0.00455)	0.00620	(0.00317)
T_{last} (h)	24.0	(7.59)	38.0	(11.8)
V_{ss} (mL/kg)	1188	(312)	930	(146)

AUC = area under the plasma concentration-time; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; CL = plasma clearance; F = female; IV = intravenous; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; PK = pharmacokinetic; SD = standard deviation; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; TDF = tenofovir disoproxil fumarate; TFV = tenofovir; V_{ss} = volume of distribution at steady state
Additional information: No statistically significant differences were observed between male and female animals in any treatment group and none were observed between fed and fasted states in the 250 mg/kg TDF dose group. Oral bioavailabilities for the 5, 50 & 250 mg/kg groups were 32.4, 23.7 & 17.0%, respectively.

4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES

4.1. BIC

The toxicokinetic profiles of BIC were examined following repeat oral administration as part of toxicology studies. Comprehensive tabulated summaries of the study results are presented in m2.6.7, Section 3.1.

4.2. FTC

4.2.1. TOX-109: Oncogenicity study of FTC in Mice – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Two-year Oral Oncogenicity Study in CD-1 Mice	Absorption	FTC	TOX-109

Species	Mouse
Feeding Condition	Fed
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Sample	Plasma
Analyte	FTC
Assay	LC-MS/MS
Dose (mg/kg/day)	0, 80, 250, 750
Sex (M/F) No. of Animals	3/sex/group on Week 2 & Week 26

Parameters	Week		Daily Dose		
			80 mg/kg	250 mg/kg	750 mg/kg
AUC_{0-24} (µg•h/mL)	**Week 2**	Female	27.59	64.97	209.16
		Male	25.52	91.05	234.58
		Mean	26.56	78.01	221.87
	Week 26	Female	23.74	91.71	322.65
		Male	27.48	90.94	287.3
		Mean	25.61	91.33	304.98
C_{max} (µg/mL)	**Week 2**	Female	10.446	33.373	109.690
		Male	16.200	46.609	88.693
		Mean	13.323	39.991	99.192
	Week 26	Female	20.487	49.058	176.950
		Male	16.265	57.682	143.229
		Mean	18.372	53.370	160.090

AUC_{0-24} = area under the plasma concentration-time curve from zero to 24 h; C_{max} = maximum plasma concentration; F = female; FTC = emtricitabine; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male

4.2.2. TOX-108: Oncogenicity Study of FTC in Rats – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Two-year Oral Oncogenicity Study of FTC in the Rat	Absorption	FTC	TOX-108

Species	Rat
Feeding Condition	Fed
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Sample	Plasma
Analyte	FTC
Assay	LC-MS/MS
Dose (mg/kg/day)	0, 60, 200, 600
Sex (M/F) No. of Animals	3/sex/group on Week 2 & Week 26

Parameters	Week		Daily Dose		
			60 mg/kg	200 mg/kg	600 mg/kg
AUC_{0-24} (µg•h/mL)	**Week 2**	Female	30.91	155.99	260.02
		Male	29.91	97.26	279.68
		Mean	30.41	126.62	269.85
	Week 26	Female	52.53	170.68	404.07
		Male	42.87	137.42	326.77
		Mean	47.70	154.05	365.42
C_{max} (µg/mL)	**Week 2**	Female	11.044	30.991	63.813
		Male	12.380	27.565	59.610
		Mean	11.712	29.278	61.712
	Week 26	Female	15.569	52.168	88.993
		Male	13.996	32.339	73.053
		Mean	14.783	42.254	81.023

AUC_{0-24} = area under the plasma concentration-time curve from zero to 24 h; C_{max} = maximum plasma concentration; F = female; FTC = emtricitabine; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male

4.3. TAF

4.3.1. AD-120-2033: 7-Day Repeated Dose Study of TAF in Dogs – Pharmacokinetics

Report Title:	Study Type	Test Article	Report Number
Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (GS-7340) Following 7-Day Oral Administration in Male Beagle Dogs	Absorption	TAF	AD-120-2033

Species: Beagle dogs

Feeding Condition: Not fasted

Vehicle/Formulation: 0.1% (w/w) hydroxypropylmethylcellulose K100LV (HPMC) K100LV, 0.1% polysorbate 20 in water

Method of Administration: Oral Gavage

Dose: 8.29 mg/kg/Day

Assay: LC-MS/MS

Test Article	**GS-7340-02**						
Sex (M/F) / N of Animals	**M/4**						
Sample	**Plasma**				**Liver**		
Sample Collection Time	**Day 1**		**Day 7**		**Day 7**		
Analyte	**TAF**	**TFV**	**TAF**	**TFV**	**TFV**	**TFV-MP**	**TFV-DP**
PK Parameters							
T_{max} (h)	0.17	0.75	0.17	0.75	4.00	4.00	4.00
C_{max} (µg/mL)	2.15	0.42	1.12	0.61	4.34	27.3	108
$t_{½}$ (h)	0.30	15.7	0.28	19.2	NA	NA	NA
AUC_{0-t} (µg•h/mL)	0.88	1.96	0.37	3.39	NA	NA	NA

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate

4.3.2. D990175-PK: 28-Day Toxicity Study of TAF in Dogs – Toxicokinetics

Report Title:	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species: Beagle dogs
Feeding Condition: Not fasted
Vehicle/Formulation: 50 mM citric acid
Method of Administration: Oral Gavage
Sample: Plasma
Analyte: TAF
Assay: LC-MS/MS

Test Article	GS-7340-02											
Dose (mg/kg/day)	1.0				3.0				10.0			
Sex (M/F) / N of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
PK Parameters												
T_{max} (h)	0.33	0.42	0.25	0.33	0.31	0.31	0.50	0.50	0.44	0.44	0.38	0.38
C_{max} (µg/mL)	0.02	0.03	0.02	0.05	0.05	0.11	0.06	0.10	0.86	2.07	0.75	1.01
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	NA	0.31	0.65	0.33	0.56
AUC_{0-t} (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.60	NA	0.41	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.30	NA	0.86
T_{last} (h)	NA	NA	NA	NA	NA	NA	NA	NA	1.50	3.00	1.00	3.00
C_{last} (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.08	0.07	0.18	0.05

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; $AUC_{0-\tau}$ = area under the plasma concentration-time curve for a dosing interval; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title:	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species: Beagle dogs
Feeding Condition: Not fasted
Vehicle/Formulation: 50 mM citric acid
Method of Administration: Oral Gavage
Sample: Plasma
Analyte: TFV
Assay: LC-MS/MS

Test Article	GS-7340-02											
Dose (mg/kg/Day)	1.0				3.0				10.0			
Sex (M/F) / N of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
PK Parameters												
T_{max} (h)	NA	3.50	NA	4.75	1.13	1.25	1.75	0.88	1.25	0.81	0.75	0.63
C_{max} (µg/mL)	NA	0.06	NA	0.07	0.12	0.09	0.15	0.13	0.38	0.55	0.40	0.43
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	NA	16.3	>24	16.6	>24
AUC_{0-t} (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	1.85	NA	1.78	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.45	NA	5.13
T_{last} (h)	NA	NA	NA	NA	NA	NA	NA	NA	21.0	24.0	18.0	24.0
C_{last} (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.03	0.15	0.05	0.16

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; $AUC_{0-\tau}$ = area under the plasma concentration-time curve for a dosing interval; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title:	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species: Beagle dogs
Feeding Condition: Not fasted
Vehicle/Formulation: 50 mM citric acid
Method of Administration: Oral Gavage
Sample: PBMC
Analyte: TFV
Assay: LC-MS/MS

Test Article	GS-7340-02					
Dose (mg/kg/Day)	**1.0**		**3.0**		**10.0**	
Sex (M/F) / N of Animals	**M/4**	**F/4**	**M/4**	**F/4**	**M/4**	**F/4**
Intracellular Concentration						
$C_{Day\ 1}$ (µg/mL)	NA	NA	NA	NA	NA	NA
$C_{Day\ 8}$ (µg/mL)	NA	NA	NA	1.80	6.92	7.85
$C_{Day\ 22}$ (µg/mL)	NA	NA	1.70	NA	10.6	10.2
$C_{Day\ 28}$ (µg/mL)	NA	NA	2.44	3.81	19.4	17.5

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

4.3.3. P2000114-PK: 28-Day Toxicity Study of TAF in Monkeys – Toxicokinetics

Report Title:	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species: Rhesus monkeys

Feeding Condition: Fasted

Vehicle/Formulation: 50 mM citric acid

Method of Administration: Nasogastric gavage

Sample: Plasma

Assay: LC-MS/MS

Test Article	**GS-7340-02**															
Dose (mg/kg/Day)	**3**								**30**							
Sex (M/F) / N of Animals	**M/3**				**F/3**				**M/3**				**F/3**			
Sample Collection Time	**Day 1**		**Day 28**		**Day 1**		**Day 28**		**Day 1**		**Day 28**		**Day 1**		**Day 28**	
Analyte	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**	**TAF**	**TFV**
PK Parameters																
T_{max} (h)	1.00	1.00	1.00	2.30	1.00	1.00	0.83	1.00	1.00	1.00	0.50	0.75	1.00	1.00	0.50	0.67
C_{max} (µg/mL)	0.003	0.09	0.01	0.05	0.005	0.09	0.02	0.05	0.49	0.96	2.21	1.24	2.29	0.85	0.82	0.78
$t_{½}$ (h)	NA	7.77	NA	12.9	NA	8.41	NA	14.2	NA	14.8	0.18	15.6	NA	10.3	0.43	16.4
AUC_{inf} (µg•h/mL)	NA	0.45	NA	NA	NA	0.49	NA	NA	NA	8.70	NA	NA	NA	4.63	NA	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	NA	NA	0.32	NA	NA	NA	0.38	NA	NA	1.53	7.36	NA	NA	0.70	3.89
$MRT_{0-\infty}$ (h)	NA	9.38	NA	16.6	NA	10.8	NA	17.4	NA	19.9	0.71	17.7	NA	12.9	2.61	20.2

$AUC_{0-\tau}$ = area under the plasma concentration-time curve for a dosing interval; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; MRT = mean residence time; NA = not applicable; PBMC = peripheral blood mononuclear cells; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title:	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species: Rhesus monkeys
Feeding Condition: Fasted
Vehicle/Formulation: 50 mM citric acid
Method of Administration: Nasogastric gavage
Sample: Plasma
Analyte: TFV
Assay: LC-MS/MS

Test Article	**TFV**			
Dose (mg/kg/Day)	**15**			
Sex (M/F) / N of Animals	**M/3**		**F/3**	
Sample Collection Time	**Day 1**	**Day 28**	**Day 1**	**Day 28**
PK Parameters				
T_{max} (h)	1.00	1.5	2.00	2.17
C_{max} (µg/mL)	0.16	0.15	0.12	0.18
$t_{½}$ (h)	6.79	17.6	11.2	12.6
AUC_{inf} (µg•h/mL)	1.01	NA	1.01	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	1.19	NA	1.46
$MRT_{0-\infty}$ (h)	8.83	21.8	16.0	16.8

$AUC_{0-\tau}$ = area under the plasma concentration-time curve for a dosing interval; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; MRT = mean residence time; NA = not applicable; PBMC = peripheral blood mononuclear cells; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title:	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species: Beagle dogs
Feeding Condition: Fasted
Vehicle/Formulation: 50 mM citric acid
Method of Administration: Nasogastric gavage
Sample: PBMC
Analyte: TFV
Assay: LC-MS/MS

Test Article	GS-7340-02				TFV	
Dose (mg/kg/Day)	**3**		**30**		**15**	
Sex (M/F) / N of Animals	**M/3**	**F/3**	**M/3**	**F/3**	**M/3**	**F/3**
Intracellular Concentrations						
C_{Day14} (µg/mL)	NA	NA	70.9	73.6	NA	NA
C_{Day28} (µg/mL)	NA	NA	31.1	24.6	NA	NA

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

5. PHARMACOKINETICS: IN VITRO AND IN VIVO DISTRIBUTION

5.1. BIC

5.1.1. AD-141-2312: In Vitro Assessment of Blood Distribution of BIC

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Blood Distribution of Bictegravir	Distribution	BIC	AD-141-2312
Study System	Heparinized blood samples from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human		
Method	BIC and control compounds was incubated in triplicate, at an initial concentration of 0.5 µM, with blood or reference plasma or reference cell fraction for 60 minutes at 37°C and then chilled on ice. Blood samples were then centrifuged at 4°C to separate the cellular and plasma fractions. Test article concentration in each fraction was determined by LC-MS/MS.		

Species	BIC[a]		Control Compound[a]	
	CPR	B/P ratio	Compound	B/P ratio
Sprague-Dawley Rat	0.05 ± 0.02	0.58 ± 0.01	Chlorthalidone	35.0 ± 6.1
Beagle Dog	0.17 ± 0.06	0.60 ± 0.03	Chloroquine	4.3 ± 0.7
Cynomolgus Monkey	0.14 ± 0.02	0.65 ± 0.01	Methazolamide	60.7 ± 13.7
Rhesus Monkey	0.11 ± 0.05	0.62 ± 0.02	Methazolamide	107.9 ± 30.0
Human	0.19 ± 0.07	0.64 ± 0.03	Methazolamide	9.0 ± 8.0

BIC = bictegravir (GS-9883); B/P ratio = Whole Blood to Plasma Concentration Ratios; CPR = Cell/Plasma Concentration Ratios; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry
a Values are the Mean ± standard deviation (n = 3 determinations using pooled blood samples from each species)

5.1.2. AD-141-2276: Distribution in Wistar Han Rats Following a Single Oral Dose of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Distribution	[^{14}C]BIC	AD-141-2276

Species	Wistar Han (non-pigmented) Rat
Gender /No. of Animals	Male / 9 (1 per time point)
Feeding Condition	Fasted
Vehicle/Formulation	5% ethanol, 55% polyethylene glycol 300, and 40% water
Method of Administration	Oral Gavage
Dose	2 mg/kg (100 µCi/kg)
Radionuclide	Carbon-14
Specific Activity	55.9 mCi/mmol
Specific Activity of Formulation	52.5 µCi/mg
Sampling Time	0.25, 1, 4, 8, 12, 24, 48, 96 and 168 h post-dose
Analyte/Assay	Carbon-14/Quantitative Whole Body Autoradiography

Tissues/Organs ↓	**Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]BIC/g tissue)**								
Time-point →	**0.25 h**	**1 h**	**4 h**	**8 h**	**12 h**	**24 h**	**48 h**	**96 h**	**168 h**
Adrenal gland(s)	ND[a]	4170	2890[b]	1360	1250	1240[b]	719	96.7[b]	246
Bile	1340	8310	6350	4160	4480	4770	1400	ND	ND
Blood	1360	18700	10000	10500	7530	6360	3640	473	709
Bone	BLQ	282	190	146	87.2	69.0	70.0	BLQ	BLQ
Bone marrow	198	3820	2250	1410	1520	1170	576	66.4	103
Brain cerebellum	BLQ	183	116	117	81.7	81.2	39.5	ND	ND
Brain cerebrum	BLQ	168	138	101	106	79.9	50.5	ND	ND
Brain medulla	BLQ	150	121	82.7	86.9	63.8	37.5	ND	ND

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Distribution	[^{14}C]BIC	AD-141-2276

Tissues/Organs ↓ / Time-point →	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]BIC/g tissue)								
	0.25 h	**1 h**	**4 h**	**8 h**	**12 h**	**24 h**	**48 h**	**96 h**	**168 h**
Brain olfactory lobe	BLQ	193	141	149	113	62.5	54.6	ND	ND
Cecum	40.4	1060	3180	1660	1480	1170	724[b]	108	159
Diaphragm	31.3	630	1160	926	776	772	431	54.4	98.3
Epididymis	27.5[b]	905[b]	1230[b]	2000[b]	1680[b]	800[b]	811[b]	67.8[b]	87.7[b]
Esophagus	ND[a]	1030	1970	2310	2160	1980	956	92.3	200
Exorbital lacrimal gland	49.2	838	1480	1460	1180	1090	457	74.2	166
Eye lens	ND	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	ND	ND
Eye uveal tract	66.4	914	1570	2740	1130	1450	655	128	170
Eye(s)	BLQ	341	614	371	288	445	229	32.7	69.5
Fat (abdominal)	29.8	404	370	285	343	140	104	BLQ	28.1
Fat (brown)	255	2810	1990	1220	923	599	483	135	131
Harderian gland	23.3	1230	1750	775	1020	627	353	59.6	78.5
Intra-orbital lacrimal gland	34.0	577	1420	1270	1420	964	629	62.0	210
Kidney cortex	199	2840	2760	1720	1720	1340	817	107	202
Kidney medulla	388	5760	4460	2620	1510	1800	1260	123	308
Kidney(s)	151	3210	2940	1940	1850	1470	872	109	213
Large intestine	ND	668	2210	2700	2780	1270	716	145	200
Liver	218	3290	2860	1430	1110	817	869	498	270
Lung(s)	523	10000	5810	5150	4410	3150	1920	249	396
Muscle	ND	351	623	758	399	433	252	33.2	55.3

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Distribution	[^{14}C]BIC	AD-141-2276

Tissues/Organs ↓ / Time-point →	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]BIC/g tissue)								
	0.25 h	**1 h**	**4 h**	**8 h**	**12 h**	**24 h**	**48 h**	**96 h**	**168 h**
Myocardium	266	5020	3760	2350	2520	1810	1150	113	239
Nasal turbinates	123	1350	1000	1470	873	561	415	86.7	109
Pancreas	71.3	1540	1600	1100	969	875b	390	59.4	107
Pituitary gland	170	2880	2160	2390	1660	1010	859	123	168
Preputial gland	ND	771[b]	1520[b]	2000[b]	718[b]	573[b]	327[b]	79.3[b]	73.9[b]
Prostate gland	47.4	648	1430	1790	896	1070	549	73.8	97.9
Salivary gland(s)	67.8	1630	2280	1400	1580	1140	604	88.1	141
Seminal vesicle(s)	ND	59.6	349	89.8	209	130	66.9[b]	BLQ	BLQ
Skin (nonpigmented)	39.1	243	1400	1670	1650	1790	958	195	266
Small intestine	129	8050	2800	908	1120	561	436	89.8	98.4
Spinal cord	BLQ	249	196	112	95.7	73.5	26.8	ND	ND
Spleen	128	1810	1290	948	767	605	390	46.2	73.4
Stomach	ND[a]	1030	1280	1590	2360	1250	888	96.2	136
Testis(es)	24.6	943	2600	1820	1300	1060	699	74.4	136
Thymus	BLQ	249	796	994	593	607	360	40.6	57.1
Thyroid	213	3730	3440	1850	1920	1460	751	137	184
Urinary bladder	61.3	905	2340	3570	3720	4480	2520	305	ND
Urine	64.3	238	281	1890	446	1450	236	95.5	ND

BIC = bictegravir (GS-9883); BLQ = below the limit of quantitation (<18.7 ng equivalents ^{14}C-GS-9883/g); h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue)
Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing
a Tissue not detected due to flare of gastrointestinal contents
b Tissue appeared to be fat soaked

5.1.3. AD-141-2276: Distribution in Long Evans Rats Following a Single Oral Dose of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Distribution	[^{14}C]BIC	AD-141-2276

Species	Long Evans (pigmented) Rat
Gender (M/F)/No. of Animals	Male / 9 (1 per time point)
Feeding Condition	Fasted
Vehicle/Formulation	5% ethanol, 55% polyethylene glycol 300, and 40% water
Method of Administration	Oral Gavage
Dose	2 mg/kg (100 µCi/kg)
Radionuclide	Carbon-14
Specific Activity	55.9 mCi/mmol
Specific Activity of Formulation	52.5 µCi/mg
Analyte/Assay	Carbon-14/Quantitative Whole Body Autoradiography

Tissues/Organs ↓	**Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]BIC/g tissue)**								
Time-point →	**0.25 h**	**1 h**	**4 h**	**8 h**	**12 h**	**24 h**	**48 h**	**96 h**	**168 h**
Adrenal gland(s)	983	2820	2750	1440	979	874[a]	978	349[a]	310[a]
Bile	5630	7430	5120	4630	2670	938	4190	ND	ND
Blood	6610	11500	12500	10300	7790	4260	4430	1860	1320
Bone	56.0	169	211	257	108	120	76.7	22.8	BLQ
Bone marrow	1140	2350	2700	1370	1320	990	683	252	161

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Distribution	[^{14}C]BIC	AD-141-2276

Tissues/Organs↓	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]BIC/g tissue)								
Time-point →	0.25 h	1 h	4 h	8 h	12 h	24 h	48 h	96 h	168 h
Brain cerebellum	105	181	161	165	129	65.6	67.5	26.9	18.9
Brain cerebrum	108	197	160	160	128	65.5	80.9	29.8	BLQ
Brain medulla	85.6	134	143	91.7	83.9	51.2	60.9	20.2	BLQ
Brain olfactory lobe	101	182	101	82.9	68.0	71.2	38.2	52.3	BLQ
Cecum	219	1760	1540	1490	1390	798	1010	494	251
Diaphragm	220	998	1060	1130	765	536	544	235	148
Epididymis	113[a]	918[a]	2000[a]	1820	2260	663[a]	NR	385	284[a]
Esophagus	ND[b]	1150	2700	2240	2020[a]	1750	1060	510	329
Exorbital lacrimal gland	142	1260	1800	1240	1320	729	831	435	217
Eye lens	BLQ	BLQ	26.4	23.3	BLQ	21.7	BLQ	ND	ND
Eye uveal tract	267	2120	3830	3650	2780	1750	1960	687	383
Eye(s)	72.8	224	552	671	372	331	271	99.1	60.5
Fat (abdominal)	78.5	376	226	184	160	176	94.6	43.8	22.7
Fat (brown)	680	1490	2320	1230	879	1230	798	240	204
Harderian gland	126	655	1850	1160	1070	648	459	252	152
Intra-orbital lacrimal gland	NR	1140	1750	1950	1370	804	816	347	174
Kidney cortex	1090	2790	2900	1930	1670	1020	1020	475	275

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Distribution	[^{14}C]BIC	AD-141-2276

Tissues/Organs ↓	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]BIC/g tissue)								
Time-point →	0.25 h	1 h	4 h	8 h	12 h	24 h	48 h	96 h	168 h
Kidney medulla	2990	5080	4720	2390	1420	1640	1710	797	448
Kidney(s)	1420	2950	3460	1900	1570	1050	1130	551	313
Large intestine	239	1580	2360	2100	2650	1300	1280	560	269
Liver	2930	4110	3690	1450	1100	775	567	491	314
Lung(s)	3500	7220	8920	5690	5080	2050	2860	1030	660
Muscle	73.6	525	514	588	416	323	348	153	101
Myocardium	1470	3500	4220	3090	2110	1190	1170	532	386
Nasal turbinates	691	1580	1300	1270	712	613	467	245	161
Pancreas	299	1400	1630	1040	895	531	643	251	157
Pituitary gland	925	2490	3010	1800	1240	1060	831	406	236
Preputial gland	101[a]	740[a]	583[a]	708	706[a]	515[a]	862	212[a]	115[a]
Prostate gland	268	973	1030	1400	1340	716	539	237	167
Salivary gland(s)	391	2760	3070	1960	1390	835	997	337	232
Seminal vesicle(s)	25.7	142	96.6	106	216	131	59.8	21.0	32.8
Skin (nonpigmented)	96.6	458	670	891	1210	1290	1340	655	350
Skin (pigmented)	186	468	948	1380	1540	1320	1300	747	385
Small intestine	375	5590	2610	1300	1280	717	527	366	212

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Distribution	[^{14}C]BIC	AD-141-2276

Tissues/Organs↓	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]BIC/g tissue)								
Time-point →	0.25 h	1 h	4 h	8 h	12 h	24 h	48 h	96 h	168 h
Spinal cord	127	249	129	153	73.5	69.1	49.5	25.1	BLQ
Spleen	671	1490	1500	1190	860	510	437	195	130
Stomach	362	1560	1500	1970	1370	941	796	255	161
Testis(es)	80.6	669	2030	1560	1110	751	1020	319	203
Thymus	116	634	875	840	855	398	566	167	110
Thyroid	949	2340	3660	2170	1740	953	1100	431	296
Urinary bladder	78.4	ND	1270	1370	1240	4100	2460	1220	582
Urine	304	ND	155	1030	151	671	104	82.4	BLQ

BIC = bictegravir (GS-9883); BLQ = below the limit of quantitation (<18.7 ng equivalents ^{14}C-GS-9883/g); h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue); NR = not represented (tissue not present in section)
Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing
a Tissue appeared to be fat soaked
b Tissue not detectable due to flare of gastrointestinal contents

5.2. FTC

5.2.1. TOX092: Tissue Distribution and Excretion Study of [^{14}C]FTC in Rats

Report Title	Study Type	Test Article	Report Number
[^{14}C]TP-0006: A Tissue Distribution and Excretion Study in Rats	Tissue Distribution, Excretion	[^{14}C]FTC	TOX092

Species	Rat
Sex (M/F) No. of Animals	20 M Sprague-Dawley group 1, 6 M Long-Evans group 2
Feeding Condition	Fasted overnight until 4 hours postdose
Vehicle/Formulation	Sterile water
Method of Administration	Oral
Dose (mg/kg)	200, single dose
Radionuclide	^{14}C
Specific Activity	42.1 mCi/mmol
Sampling Time	1, 4, 8, 24, 72 and 144 hours

	Mean Tissue:Plasma Concentration Ratios Post Dosing											
	1 hour		4 hours		8 hours		24 hours		72 hours		144 hours	
Tissues/Organs	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Blood	0.762	0.057	0.829	0.041	NA	NA	NA	NA	NA	NA	NA	NA
Large Intestine Content	0.742	0.037	1.01	0.273	15.0	7.00	NA	NA	NA	NA	NA	NA
Kidney	2.45	0.388	2.10	0.431	2.62	NA	NA	NA	NA	NA	NA	NA
Liver	1.17	0.055	1.09	0.079	NA	NA	NA	NA	NA	NA	NA	NA
Renal Cortex	2.21	0.393	2.05	0.410	2.52	NA	NA	NA	NA	NA	NA	NA
Small Intestine Content	1.27	0.359	1.31	0.596	NA	NA	NA	NA	NA	NA	NA	NA
Cerebellum	0.068	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cerebrum	0.066	0.001	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Additional Information	Data for SD rats only.											

F = female; M = male; NA= not available or not sampled.

5.3. TAF

5.3.1. AD-120-2011: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Mouse Following Oral Administration

Report Title:	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species:	CD-1 Mice
Sex (M/F) / No. of Animals:	M/30
Method of Administration:	Oral gavage
Dose (mg/kg/day):	100
Feeding Condition:	Not fasted
Specific Activity:	57.1 mCi/mmol
Radionuclide:	Carbon-14
Vehicle/Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample:	Plasma/Blood
Analyte/Assay:	[^{14}C]TAF / Liquid Scintillation Counter

Sample Type	**Plasma**	**Blood**
Tabulated PK Results (Mean)		
T_{max} (h)	0.25	0.50
C_{max} (ng eq/g)	24500	23100
$t_{½}$ (h)	15.8	45.0
AUC_{0-t} (ng eq•h/g)	108675	514848
AUC_{inf} (ng eq•h/g)	111574	NA

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; F = female; M = male; NA = not applicable; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species: CD-1 Mice

Sex (M/F) / No. of Animals: M/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Mean Concentrations Postdosing (ng Equivalents [^{14}C]TAF/g)				
Time Point	**Brain**	**Heart**	**Kidney**	**Liver**	**Nasal Turbinates**
1 h	604	7660	106000	519000	5680
4 h	183	2440	67500	394000	1750
12 h	198	4290	47900	296000	1870
24 h	219	4870	26900	194000	1540
48 h	159	3550	13400	83600	1170
72 h	88.9	1460	5820	55700	546

F = female; M = male; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species: CD-1 Mice

Sex (M/F) / No. of Animals: M/9

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sampling Time: 0.5, 1, 3, 8, 12, 24, 48, 96, and 168 hours postdose

Analyte/Assay: [^{14}C]TAF / Quantitative whole body autoradiography

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)								
	Animal Number (Sacrifice Time)								
Tissue	**A18225 0.5h**	**A18226 1 h**	**A18227 3 h**	**A18228 8 h**	**A18229 12 h**	**A18230 24 h**	**A18231 48 h**	**A18232 96 h**	**A18233 168 h**
Blood and Tissue Radioactivity									
Adrenal gland(s)	9920	27500	6450	5420	3010	3600	2550	1530	669
Bile	60300	127000	115000	NR	65900	12900	12200	5940	NR
Blood	14600	14500	5280	8640	4380	6100	4100	2710	1000
Bone	1300	2420	1230	1140	1010	525	BLQ	372	ND
Bone marrow	4730	6610	2030	1670	2040	2120	1190	1140	513
Brain cerebellum	569	1940	395	BLQ	BLQ	BLQ	BLQ	ND	ND

Report Title: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	**Study Type** Distribution	**Test Article** [^{14}C]TAF	**Report Number** AD-120-2011

Blood and Tissue Radioactivity (continued)

Brain cerebrum	768	1430	BLQ	BLQ	379	BLQ	BLQ	ND	ND
Brain medulla	420	833	BLQ	BLQ	BLQ	BLQ	BLQ	ND	ND
Brain olfactory lobe	1120	2890	464	398	461	BLQ	BLQ	ND	ND
Cecum	3440	6960	90500	63200	19100	8310	2290	2540	1310
Diaphragm	40600	167000	46600	82900	26600	28300	19000	10300	3320
Epididymis	3600	22300	1310	508	540	864	BLQ	ND	ND
Esophagus	38400	90800	57300	114000	20100	8660	8660	5860	933
Exorbital lacrimal gland	3600	5460	1400	1840	1250	1290	748	729	364
Eye lens	967	2980	508	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Eye uveal tract	4180	3690	754	488	947	779	BLQ	ND	ND
Eye(s)	1880	2660	539	312	418	363	BLQ	BLQ	ND
Fat (abdominal)	1250	1170	968	757	512	787	BLQ	ND	ND
Fat (brown)	3070	5590	2530	3790	2060	3030	1920	1060	BLQ
Gall bladder	335000	216000	108000	NR	68100	37100	20900	29200	NR
Harderian gland	4240	6500	1530	2050	2170	1390	764	1210	487
Intra-orbital lacrimal gland	7630	3580	1900	1190	2280	1500	NR	ND	442
Kidney cortex	82300	89000	74000	37400	30000	23100	10500	7300	2830
Kidney medulla	65000	70000	54800	29600	18100	12800	7070	4430	1670

Report Title: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	**Study Type** Distribution	**Test Article** [^{14}C]TAF	**Report Number** AD-120-2011

Blood and Tissue Radioactivity (continued)

Kidney(s)	84800	86100	68900	3370	25900	19900	9090	6080	2300
Large intestine	6680	8090	7730	96100	22500	6770	3100	2200	607
Liver	282000	447000	290000	295000	197000	164000	77000	44600	19000
Lung(s)	13200	23900	4000	8900	10000	11500	6890	5220	1820
Lymph node(s)	2190	7750	1680	1690	1760	2280	1470	2090	468
Muscle	1680	2100	466	522	768	579	363	366	BLQ
Myocardium	7570	11700	2380	4100	4600	4620	3430	3500	905
Nasal turbinates	2720	6440	1290	1170	983	1190	519	709	370
Pancreas	6130	14500	2950	2970	3540	2880	1760	1970	917
Pituitary gland	2910	7440	1280	1390	2510	1300	663	718	BLQ
Preputial gland	1900	2580	635	390	708	503	BLQ	ND	ND
Prostate gland	5500	2880	2150	16500	3060	2590	427	440	ND
Salivary gland(s)	5390	11500	1880	2210	2420	2160	1130	907	511
Seminal vesicle(s)	1790	3410	957	27000	852	620	462	BLQ	ND
Skin (pigmented)	8520	5580	1510	939	674	715	368	BLQ	ND
Small intestine	8770	74500	88100	54900	14100	4040	3400	3520	1610
Spinal cord	757	1210	BLQ	437	BLQ	BLQ	BLQ	ND	ND
Spleen	6100	12900	3850	4700	5460	6270	4020	3040	1430

Report Title: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	**Study Type** Distribution	**Test Article** [^{14}C]TAF	**Report Number** AD-120-2011

Blood and Tissue Radioactivity (continued)

Stomach	77600	39300	26600	49700	5460	5940	5870	1760	571
Stomach mucosa	96900	46900	19800	44000	5280	3660	5910	2550	638
Stomach wall	48600	24500	26600	24700	7690	22700	4230	3940	378
Testis(es)	1160	1490	774	BLQ	BLQ	319	ND	ND	ND
Thymus	2510	6140	914	1300	903	1190	808	789	BLQ
Thyroid	7120	12100	2830	2290	1200	4600	3200	3500	1530
Urinary bladder	ND	174000	85600	ND	5800	10300	9240	2800	924
Urine	1790000	413000	200000	351000	36800	24700	14600	327	BLQ

BLQ = below the limit of quantitation (< 311 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species: C57 Black Mice

Sex (M/F) / No. of Animals: M/9

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sampling Time: 0.5, 1, 3, 12, 24, 96, 168, 240, and 336 hours postdose

Analyte/Assay: [^{14}C]TAF / Quantitative whole body autoradiography

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
Tissue	**A19068 0.5h**	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Blood and Tissue Radioactivity									
Adrenal gland(s)	34000	26400	20400	7750	10400	2710	2310	1610	1430
Bile	198000	136000	150000	40300	26600	3620	1020	2360	2160
Blood	16700	9270	7910	4170	3780	1100	788	675	BLQ
Bone	11200	4490	4900	2430	1770	ND	ND	ND	ND
Bone marrow	24300	19000	16100	6440	6470	ND	ND	ND	ND
Brain cerebellum	611	590	BLQ	ND	ND	ND	ND	ND	ND

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	A19068 0.5h	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Blood and Tissue Radioactivity (continued)									
Brain cerebrum	1240	661	BLQ	ND	ND	ND	ND	ND	ND
Brain medulla	886	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	3790	1730	1640	688	707	ND	ND	ND	ND
Cecum	25400	95400	ND	50500	10500	BLQ	ND	ND	ND
Diaphragm	60300	103000	114000	27500	25900	2340	2360	1830	1410
Epididymis	10800	4190	4990	2120	2150	ND	ND	ND	ND
Esophagus	76100	81300	48900	27900	8540	1540	1660	723	ND
Exorbital lacrimal gland	18100	12000	11800	4910	3530	1050	1010	940	ND
Eye lens	4310	1870	1110	527	BLQ	ND	ND	ND	ND
Eye uveal tract	13400	12200	11600	4740	6140	ND	ND	ND	ND
Eye(s)	5000	2920	2440	1040	1060	ND	ND	ND	ND
Fat (abdominal)	5460	6820	2140	4180	1700	6410	BLQ	ND	ND
Fat (brown)	19200	18700	14500	10800	8660	2420	1190	812	569
Gall bladder	163000	379000	275000	94000	39800	6580	4130	ND	ND
Harderian gland	18500	14600	13600	6200	4580	861	ND	ND	ND
Intra-orbital lacrimal gland	NR	NR	NR	NR	3760	ND	ND	ND	ND
Kidney cortex	137000	125000	104000	58000	34500	5660	5350	4300	2530
Kidney medulla	125000	94700	80000	40200	19400	3640	2980	2620	1820

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	A19068 0.5h	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Blood and Tissue Radioactivity (continued)									
Kidney(s)	132000	107000	89600	47500	29500	4820	4540	3550	2240
Large intestine	25700	17300	32300	74200	35000	2930	1530	785	BLQ
Liver	488000	490000	385000	282000	118000	21900	14200	9840	7510
Lung(s)	32300	32500	26500	18100	13500	4440	4260	3330	2120
Lymph node(s)	17900	14600	12600	8020	10900	ND	ND	ND	ND
Muscle	8780	4140	3190	2010	3900	753	982	650	491
Myocardium	23200	13500	13600	8470	9510	2490	2170	2070	1540
Nasal turbinates	10600	2980	3550	2390	1920	799	ND	ND	ND
Pancreas	34900	26900	31500	15400	13900	1780	1940	1650	1220
Pituitary gland	17400	8440	6590	850	ND	ND	ND	ND	ND
Preputial gland	11500	6390	6130	2240	1970	ND	ND	ND	ND
Prostate gland	ND	8630	4840	8620	ND	ND	ND	ND	ND
Salivary gland(s)	36200	24200	27200	9910	12200	1150	1080	899	522
Seminal vesicle(s)	5540	3530	2470	2150	825	BLQ	ND	ND	ND
Skin (pigmented)	11600	5930	3350	1180	1340	ND	ND	ND	ND
Small intestine	26900	56100	28000	14100	11300	5030	ND	ND	ND
Spinal cord	1950	742	BLQ	BLQ	ND	ND	ND	ND	ND
Spleen	35300	29600	29100	16600	12200	2570	2560	1550	978

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	A19068 0.5h	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Blood and Tissue Radioactivity (continued)									
Stomach	60500	70600	29200	7900	6730	1610	1500	1230	859
Stomach mucosa	ND	ND	ND	ND	ND	ND	ND	ND	ND
Stomach wall	ND	ND	ND	ND	ND	ND	ND	ND	ND
Testis(es)	2640	1760	1070	512	752	ND	ND	ND	ND
Thymus	12400	8190	7190	3440	3410	BLQ	ND	ND	ND
Thyroid	40700	32700	29000	NR	10900	4840	4150	3160	2620
Urinary bladder	ND	138000	49100	12000	6290	1290	ND	ND	ND
Urine	1170000	626000	128000	135000	18400	495	ND	ND	ND

BLQ = below the limit of quantitation (< 490 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species: CD-1 Mice

Sex (M/F) / No. of Animals: M/4

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of Dose)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-12 h	9.75	NA	NA	9.75
0-24 h	19.3	31.1	3.95	54.4
0-48 h	24.6	36.1	4.91	65.6
0-72 h	25.9	37.6	5.50	69.0
0-96 h	26.7	39.4	6.06	72.2
0-120 h	27.1	40.4	6.40	73.9
0-144 h	27.4	41.0	6.65	75.1
0-168 h	27.7	41.3	NA	69.0

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

5.3.2. AD-120-2020: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Rat Following Oral Administration

Report Title:	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Species: Sprague-Dawley Rats

Sex (M/F) / No. of Animals: M/15

Method of Administration: Oral gavage

Dose (mg/kg/day): 5

Feeding Condition: Fasted

Specific Activity: 57.0 mCi/mmol

Radionuclide: Carbon-14

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sample: Plasma/Blood

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

Sample Type	**Plasma**	**Blood**
Tabulated PK Results (Mean)		
T_{max} (h)	0.25	0.25
C_{max} (ng eq/g)	1110	603
$t_{½}$ (h)	14.1	21.5
AUC_{0-t} (ng eq•h/g)	3591	2432
AUC_{inf} (ng eq•h/g)	3870	2588

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; F = female; M = male; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Species: Sprague-Dawley Rats

Sex (M/F) / No. of Animals: M/9

Method of Administration: Oral gavage

Dose (mg/kg): 5

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 57.0 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sampling Time: 0.25, 1, 4, 8, 12, 24, 48, 96, and 168 hours postdose

Analyte/Assay: [^{14}C]TAF / Quantitative whole body autoradiography

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
Tissue	**B35426 0.25h**	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Blood and Tissue Radioactivity									
Adrenal gland(s)	181	129	BLQ	ND	ND	ND	ND	ND	ND
Arterial wall	817	299	118	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1070	334	138	76.6	83.1	ND	ND	ND	ND
Bone	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	233	125	72.6	BLQ	BLQ	ND	ND	ND	ND

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35426 0.25h	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Blood and Tissue Radioactivity (continued)									
Brain cerebellum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	45.7	BLQ	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	396	177	236	BLQ	ND	ND	ND	ND	ND
Cecum	218	132	541	313	NR	323	261	ND	ND
Diaphragm	210	145	52.9	BLQ	ND	ND	ND	ND	ND
Epididymis	249	101	BLQ	BLQ	ND	ND	ND	ND	ND
Esophagus	341	222	187	79.8	58.9	ND	ND	ND	ND
Exorbital lacrimal gland	234	101	51.2	ND	ND	ND	ND	ND	ND
Eye lens	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	409	187	78.4	ND	ND	ND	ND	ND	ND
Eye vitreous humor	84.9	89.3	BLQ	ND	ND	ND	ND	ND	ND
Eye(s)	86.1	92.7	BLQ	ND	ND	ND	ND	ND	ND
Fat (abdominal)	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	200	97.1	48.2	BLQ	55.7	ND	ND	ND	ND
Harderian gland	98.6	52.4	BLQ	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	250	118	BLQ	ND	ND	ND	ND	ND	ND

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35426 0.25h	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Blood and Tissue Radioactivity (continued)									
Kidney cortex	10700	12400	11800	6410	8300	2010	677	70.3	ND
Kidney medulla	8240	5040	2800	1210	1010	317	161	BLQ	ND
Kidney(s)	9520	8710	8250	4720	4590	1380	511	52.0	ND
Large intestine	364	140	55.8	BLQ	ND	ND	ND	ND	ND
Liver	6730	6730	4010	2410	3570	1090	751	145	195
Lung(s)	592	211	81.1	ND	ND	ND	ND	ND	ND
Lymph node(s)	318	ND	ND	ND	ND	ND	ND	ND	ND
Muscle	101	BLQ	ND	ND	ND	ND	ND	ND	ND
Myocardium	361	136	58.6	BLQ	ND	ND	ND	ND	ND
Nasal turbinates	123	83.8	52.8	ND	ND	ND	ND	ND	ND
Pancreas	215	120	68.9	46.1	52.0	ND	ND	ND	ND
Pituitary gland	335	116	50.5	BLQ	76.2	ND	ND	ND	ND
Preputial gland	140	60.1	BLQ	ND	ND	ND	ND	ND	ND
Prostate gland	134	88.6	117	BLQ	BLQ	ND	ND	ND	ND
Salivary gland(s)	292	119	BLQ	ND	ND	ND	ND	ND	ND
Seminal vesicle(s)	62.7	ND	ND	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	393	123	BLQ	ND	ND	ND	ND	ND	ND
Small intestine	479	500	364	284	86.5	171	58.3	ND	ND

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35426 0.25h	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Blood and Tissue Radioactivity (continued)									
Spinal cord	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	147	105	59.0	BLQ	62.8	BLQ	ND	ND	ND
Stomach	475	126	66.6	ND	ND	ND	ND	ND	ND
Testis(es)	98.2	50.9	BLQ	ND	ND	ND	ND	ND	ND
Thymus	91.2	68.1	BLQ	ND	ND	ND	ND	ND	ND
Thyroid	324	147	63.6	ND	ND	ND	ND	ND	ND
Tooth pulp	646	228	84.4	ND	ND	ND	ND	ND	ND
Urinary bladder	925	205	ND	BLQ	1050	BLQ	ND	ND	ND
Urine	112000	34400	51100	3260	14500	988	645	88.3	ND

BLQ = below the limit of quantitation (< 45.6 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented (tissue not present in section); TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Species: Long Evans rats

Sex (M/F) / No. of Animals: M/9

Method of Administration: Oral gavage

Dose (mg/kg): 5

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 57.0 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sampling Time: 0.25, 1, 4, 8, 12, 24, 48, 96, and 168 hours postdose

Analyte/Assay: [^{14}C]TAF / Quantitative whole body autoradiography

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
Tissue	**B35435 0.25h**	**B35436 1 h**	**B35437 4 h**	**B35438 8 h**	**B35439 12 h**	**B35440 24 h**	**B35441 48 h**	**B35442 96 h**	**B35443 168 h**
Blood and Tissue Radioactivity									
Adrenal gland(s)	353	115	48.5	ND	ND	ND	ND	ND	ND
Arterial wall	1350	270	90.4	93.8	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1260	221	116	125	117	ND	ND	ND	ND
Bone	50.4	BLQ	ND	ND	ND	ND	ND	ND	ND
Bone marrow	311	84.9	BLQ	62.4	BLQ	ND	ND	ND	ND

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35435 0.25h	**B35436 1 h**	**B35437 4 h**	**B35438 8 h**	**B35439 12 h**	**B35440 24 h**	**B35441 48 h**	**B35442 96 h**	**B35443 168 h**
Blood and Tissue Radioactivity (continued)									
Brain cerebellum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	57.2	51.0	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	831	209	ND	ND	ND	ND	ND	ND	ND
Cecum	603	118	889	462	362	494	101	BLQ	ND
Diaphragm	353	124	55.8	BLQ	ND	ND	ND	ND	ND
Epididymis	516	79.3	BLQ	58.4	ND	ND	ND	ND	ND
Esophagus	923	218	186	637	65.8	ND	ND	ND	ND
Exorbital lacrimal gland	353	56.1	BLQ	BLQ	ND	ND	ND	ND	ND
Eye lens	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	555	89.5	59.4	BLQ	ND	ND	ND	ND	ND
Eye vitreous humor	139	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Eye(s)	150	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Fat (abdominal)	70.0	BLQ	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	232	BLQ	ND	ND	ND	ND	ND	ND	ND
Harderian gland	209	BLQ	ND	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	402	53.4	49.1	ND	ND	ND	ND	ND	ND

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35435 0.25h	**B35436 1 h**	**B35437 4 h**	**B35438 8 h**	**B35439 12 h**	**B35440 24 h**	**B35441 48 h**	**B35442 96 h**	**B35443 168 h**
Blood and Tissue Radioactivity (continued)									
Kidney cortex	8000	8890	6980	7360	5150	2440	570	65.6	ND
Kidney medulla	6900	3670	655	1920	757	367	117	BLQ	ND
Kidney(s)	7750	7570	5160	5260	3000	1310	390	48.1	ND
Large intestine	548	91.5	474	97.5	133	132	575	166	ND
Liver	10300	7800	7710	6670	5610	1380	671	221	139
Lung(s)	854	145	67.2	76.5	66.3	BLQ	ND	ND	ND
Lymph node(s)	422	94.9	ND	ND	ND	ND	ND	ND	ND
Muscle	115	BLQ	ND	ND	ND	ND	ND	ND	ND
Myocardium	512	46.6	BLQ	72.7	BLQ	ND	ND	ND	ND
Nasal turbinates	201	71.9	BLQ	ND	ND	ND	ND	ND	ND
Pancreas	274	82.1	53.5	71.0	ND	ND	ND	ND	ND
Pituitary gland	434	53.1	ND	ND	ND	ND	ND	ND	ND
Preputial gland	247	67.9	ND	ND	ND	ND	ND	ND	ND
Prostate gland	247	BLQ	49.9	ND	ND	ND	ND	ND	ND
Salivary gland(s)	368	85.3	BLQ	BLQ	BLQ	ND	ND	ND	ND
Seminal vesicle(s)	67.7	BLQ	ND	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	526	71.8	BLQ	ND	ND	ND	ND	ND	ND
Skin (pigmented)	623	78.9	BLQ	ND	ND	ND	ND	ND	ND

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35435 0.25h	**B35436 1 h**	**B35437 4 h**	**B35438 8 h**	**B35439 12 h**	**B35440 24 h**	**B35441 48 h**	**B35442 96 h**	**B35443 168 h**
Blood and Tissue Radioactivity (continued)									
Small intestine	530	379	122	278	220	98.6	BLQ	ND	ND
Spinal cord	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Spleen	231	83.1	58.3	75.8	60.7	BLQ	BLQ	ND	ND
Stomach	682	113	71.4	69.9	159	BLQ	BLQ	ND	ND
Testis(es)	157	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND
Thymus	181	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Thyroid	412	59.1	ND	ND	ND	ND	ND	ND	ND
Tooth pulp	793	194	78.7	89.3	57.0	NR	ND	ND	ND
Urinary bladder	352	155	125	89.1	45.9	48.3	BLQ	ND	ND
Urine	50200	61000	2280	6540	1140	1500	109	BLQ	ND

BLQ = below the limit of quantitation (< 45.6 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented (tissue not present in section); TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Species: Sprague-Dawley rats
Sex (M/F) / No. of Animals: M/3
Method of Administration: Oral gavage
Dose (mg/kg): 5
Feeding Condition: Fasted
Radionuclide: Carbon-14
Specific Activity: 57.0 mCi/mmol
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of Dose)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-8 h	14.6 ± 3.78	NA	NA	14.6 ± 3.78
0-24 h	19.8 ± 4.96	66.8 ± 6.14	1.22 ± 0.16	87.9 ± 1.06
0-48 h	21.5 ± 5.45	71.1 ± 4.73	1.46 ± 0.08	94.1 ± 0.71
0-72 h	21.8 ± 5.45	71.6 ± 4.50	1.56 ± 0.15	95.0 ± 1.01
0-96 h	22.0 ± 5.48	71.8 ± 4.52	1.67 ± 0.26	95.4 ± 0.97
0-120 h	22.1 ± 5.47	71.8 ± 4.51	1.70 ± 0.28	95.6 ± 0.90
0-144 h	22.1 ± 5.47	71.9 ± 4.51	1.71 ± 0.28	95.7 ± 0.95
0-168 h	22.2 ± 5.42	71.9 ± 4.49	NA	94.1 ± 0.93

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Species: Bile Duct-Cannulated SD rats
Sex (M/F) / No. of Animals: M/5
Method of Administration: Oral gavage
Dose (mg/kg): 5
Feeding Condition: Fasted
Radionuclide: Carbon-14
Specific Activity: 57.0 mCi/mmol
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of Dose)				
Time Point	**Urine**	**Feces**	**Bile**	**Cage Rinse**	**Total**
0-2 h	NA	NA	1.81 ± 0.76	NA	1.81 ± 0.76
0-4 h	NA	NA	1.93 ± 0.76	NA	1.93 ± 0.76
0-6 h	NA	NA	1.97 ± 0.77	NA	1.97 ± 0.77
0-8 h	15.3 ± 1.82	NA	2.00 ± 0.78	NA	17.3 ± 2.18
0-12 h	NA	NA	2.04 ± 0.79	NA	2.04 ± 0.80
0-24 h	21.1 ± 3.42	56.7 ± 15.3	2.09 ± 0.81	0.91 ± 0.17	80.7 ± 11.4
0-48 h	22.7 ± 3.95	70.9 ± 7.10	2.11 ± 0.82	1.14 ± 0.21	96.8 ± 2.58
0-72 h	23.0 ± 4.06	72.3 ± 6.02	2.11 ± 0.82	1.28 ± 0.27	98.6 ± 1.49
0-96 h	23.1 ± 4.11	72.4 ± 5.95	2.11 ± 0.82	1.33 ± 0.29	99.0 ± 1.40
0-120 h	23.2 ± 4.12	72.5 ± 5.96	2.11 ± 0.82	1.35 ± 0.29	99.1 ± 1.40
0-144 h	23.2 ± 4.13	72.5 ± 5.96	2.11 ± 0.82	1.37 ± 0.30	99.2 ± 1.49
0-168 h	23.2 ± 4.15	72.6 ± 5.96	2.11 ± 0.82	NA	97.9 ± 1.35

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

5.3.3. AD-120-2009: Distribution of [^{14}C]TAF in Dog Following Oral Administration

Report Title:	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Species:	Beagle dogs
Sex (M/F) / No. of Animals:	M/10
Method of Administration:	Oral gavage
Dose Administration:	Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5
Dose (mg/kg/day):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	57.1 mCi/mmol
Vehicle/Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF / Liquid Scintillation Counter

	Mean Concentration (ng Equivalents [^{14}C]GS-7340/g)	
Time Point	**Blood**	**Plasma**
1 h	2220	3180
2 h	1290	1750
6 h	811	783
12 h	449	363
24 h	340	277

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide
Note: Mean concentrations were calculated from 2 animals/timepoint.

Report Title:	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Species: Beagle dogs

Sex (M/F) / No. of Animals: M/10

Method of Administration: Oral gavage

Dose (mg/kg): 15

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Mean Concentration (ng Equivalents [^{14}C]GS-7340/g)	
Time Point	**Blood**	**Plasma**
1 h	485	778
2 h	1280	1890
6 h	401	355
12 h	295	262
24 h	201	172

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide
Note: Mean concentrations were calculated from 2 animals/timepoint.

Report Title:	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Species: Beagle dogs

Sex (M/F) / No. of Animals: M/10

Method of Administration: Oral gavage

Dose Administration: Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5

Dose (mg/kg/day): 15

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					
Time Point	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	2390	3450	45500	74900	8660	15200
2 h	4410	4420	89300	105000	13000	26500
6 h	5600	5310	162000	109000	15000	30500
12 h	4350	4330	144000	84600	9640	21600
24 h	2990	3970	107000	78100	8610	16800

F = female; M = male; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Species: Beagle dogs

Sex (M/F) / No. of Animals: M/10

Method of Administration: Oral gavage

Dose (mg/kg): 15

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					
Time Point	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	476	776	17000	47900	2480	2960
2 h	2440	2750	82500	97600	10200	12700
6 h	2600	3070	99700	97000	8870	15600
12 h	2220	2820	91800	78300	6970	16800
24 h	1100	1990	88500	64100	5260	4900

F = female; M = male; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Species: Beagle dogs

Sex (M/F) / No. of Animals: M/10

Method of Administration: Oral gavage

Dose Administration: Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5

Dose (mg/kg/day): 15

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Percent of Radioactive Dose (% of Dose)						
Time Point	**Blood**	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	0.64	1.48	0.19	1.39	15.4	0.50	0.013
2 h	0.37	2.70	0.24	2.78	23.1	0.75	0.019
6 h	0.23	3.45	0.26	4.17	19.4	0.87	0.020
12 h	0.13	2.69	0.24	3.93	18.3	0.60	0.011
24 h	0.10	1.85	0.22	2.76	15.0	0.50	0.008

F = female; M = male; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Species: Beagle dogs

Sex (M/F) / No. of Animals: M/10

Method of Administration: Oral gavage

Dose (mg/kg): 15

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 57.1 mCi/mmol

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Percent of Radioactive Dose (% of Dose)						
Time Point	**Blood**	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	0.14	0.30	0.05	0.60	8.78	0.19	0.002
2 h	0.37	1.51	0.17	2.84	19.6	0.62	0.009
6 h	0.12	1.61	0.17	3.46	18.6	0.50	0.009
12 h	0.08	1.37	0.15	3.02	18.2	0.44	0.012
24 h	0.06	0.67	0.10	2.79	13.9	0.34	0.003

F = female; M = male; TAF = tenofovir alafenamide

5.3.4. D990173-BP: Distribution of [^{14}C]TAF in Dog Following Oral Administration

Report Title:	Study Type	Test Article	Report Number
Analysis of Data from [redacted] Biosafety Study G545 "Tissue Distribution of [^{14}C]GS-7340 in Beagle Dogs Following Oral Administration"	Distribution	[^{14}C]TAF	D990173-BP

Species: Beagle dogs

Sex (M/F) / No. of Animals: M/2

Method of Administration: Oral gavage

Dose (mg/kg): 18.1

Feeding Condition: Fasted

Radionuclide: Carbon-14

Specific Activity: 53 mCi/mmol

Vehicle/Formulation: 50 mM citric acid

Sampling Time: 24 hours postdose

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Distribution of Radioactivity		
Tissue/Fluid	**Percent of Total Dose (%)**	**Percent of Recovered Dose[a] (%)**	**Concentration (µg eq TFV/g)**
Liver	16.5	26.0	52.9
Kidney	3.78	5.98	80.2
Mesenteric Lymph Nodes	0.04	0.06	6.88
Spleen	0.17	0.27	8.13
Femoral Bone	0.00	0.00	0.28
Bone Marrow	0.00	0.00	2.05
Stomach	0.26	0.41	2.68
Jejunum	0.79	1.26	4.16
Duodenum	0.44	0.70	8.77

Tissue/Fluid	Distribution of Radioactivity		
	Percent of Total Dose (%)	Percent of Recovered Dose[a] (%)	Concentration (µg eq TFV/g)
Ileum	0.16	0.26	4.61
Large Intestine	2.66	4.28	47.2
Bile	0.23	0.35	40.5
Feces	0.19	0.15	480
Stomach Content	0.04	0.06	0.91
Small Intestine Content	1.25	2.00	11.3
Large Intestine Content	20.4	32.4	155
Urine	14.7	23.1	25.2
Plasma	0.00	0.00	0.20
PBMCs	0.09	0.14	63.2[b]

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir
a Selected tissues reported
b Estimated based on the mean reported single cell volume of 0.2 picoliters

5.3.5. AD-120-2044: TAF Penetration into Cerebrospinal Fluid

Report Title:	Study Type	Test Article	Report Number
Tenofovir Alafenamide Penetration into Cerebrospinal Fluid Following a Single Oral Dose of TAF Alone or in Combination of Cobicistat in Cynomolgus Monkeys	Distribution	TAF/COBI	AD-120-2044

Species:	Cynomolgus monkeys
Feeding Condition:	Fasted
Vehicle/Formulation:	0.1% (w/w) hydroxypropylmethylcellulose K100LV (HPMC) K100LV, 0.1% polysorbate 20 in water
Method of Administration:	Oral gavage[a]
Assay:	LC-MS/MS

Test Article	**TAF & TAF+COBI**				
Sex (M/F) / N of Animals	**M/6**				
Dose	**5 mg/kg TAF**		**2 mg/kg TAF + 30 mg/kg COBI**		
Analyte	**TAF**	**TFV**	**TAF**	**TFV**	**COBI**
PK Parameters					
C_{max} (µM)	0.36 ± 0.14	0.47 ± 0.24	0.32 ± 0.26	0.33 ± 0.10	8.75 ± 1.18
T_{max} (h)	0.33 ± 0.14	0.83 ± 0.29	0.33 ± 0.14	0.83 ± 0.29	4.00 ± 0.00
AUC_{0-t} (µM·h)	0.18 ± 0.13	2.37 ± 0.46	0.23 ± 0.08	2.75 ± 0.69	71.9 ± 12.1
$t_{1/2}$ (h)	0.17 ± 0.09	8.78 ± 1.07	0.56 ± 0.42	9.33 ± 1.49	1.95 ± 0.12
CSF Concontration (µM)	BLQ[a]	BLQ	BLQ	BLQ	-

AUC_{0-t} = area under the plasma concentration-time curve from zero to last measured time-point; C_{max} = maximum plasma concentration; COBI = cobicistat; CSF = cerebrospinal fluid; F = female; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; M = male; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir
a Below lower limit of quantification. Lower limit of quantification for TAF and TFV was 7 nM.

6. PHARMACOKINETICS: PLASMA PROTEIN BINDING

6.1. BIC

6.1.1. AD-141-2287: Plasma Protein Binding of BIC in Rats, Dogs, Monkeys, and Humans

Report Title	Study Type	Test Article	Report Number
In Vitro Protein Binding Determination of GS-9883 by Equilibrium Dialysis	Distribution	BIC	AD-141-2287
Study System	Plasma from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey and human		
Method	Plasma (1 mL) containing BIC (2 µM) and compound-free phosphate buffer (1 mL) were placed into opposite sides of the assembled dialysis cells separated by a semipermeable membrane. The dialysis was carried out at 37°C for 3 hours. BIC concentrations in each cell was determined by LC-MS/MS.		
Species	**BIC Bound (%)[a]**	**BIC Unbound (%)[a]**	
Sprague-Dawley Rat	99.99 ± 0.00	0.01 ± 0.00	
Beagle Dog	98.76 ± 0.06	1.24 ± 0.06	
Cynomolgus Monkey	99.69 ± 0.01	0.31 ± 0.01	
Rhesus Monkey	99.68 ± 0.02	0.32 ± 0.02	
Human	99.75 ± 0.01	0.25 ± 0.01	
Study System:	Human plasma and CCM		
Method:	CCM and human plasma (1 mL) containing BIC (2 µM) were placed in opposite sides of the assembled dialysis cells separated by a semipermeable membrane. The dialysis was carried out at 37°C for 24 hours. BIC concentration in each cell was determined by LC-MS/MS.		
Human Plasma to CCM Ratio[a]	43.6 ± 7.7		

BIC = bictegravir (GS-9883); CCM = cell culture medium (RPMI media1640) containing 10% fetal bovine serum; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry
a Values are the mean ± standard deviation (n = 3)

6.1.2. AD-141-2311: Microsomal Binding of BIC

Report Title	Study Type	Test Article	Report Number
Human Hepatic Microsomal Binding of Bictegravir	Distribution	BIC	AD-141-2311
Method	Pooled human hepatic microsomal fraction (0.5 mg protein/mL) containing BIC (3 μM) or positive control (amitriptyline, 3 μM) was dialyzed in duplicate against buffer at 37°C for overnight. Test compound concentrations in the buffer and microsomal fraction were determined by LC-MS/MS.		

Test Compound	Fraction Unbound (%)[a]	Recovery (%)[a]
BIC	86.3	80.9
Amitriptyline	25.0	71.9

BIC = bictegravir (GS-9883); LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry
a Mean (n = 2)

6.2. FTC

6.2.1. TBZZ/93/0025: Protein Binding of FTC in Mouse, Rabbit, Monkey and Human Plasma

Report Title	Study Type	Test Article	Report Number
Protein Binding of 524W91 in Human, Monkey, Mouse, and Rabbit Plasma	Plasma Protein Binding	FTC	TBZZ/93/0025

Study system: In vitro

Target Entity, Test System, and Methods: Plasma, Equilibrium dialysis

Species	FTC concentration		% Bound
	µg/mL	µM	
Human	0.020	0.08	3.3
	0.101	0.41	0.8
	0.501	2.03	2.7
	2.51	10.2	2.2
	10.0	40.4	3.4
	49.9	202	2.0
	200	808	0.0
Monkey	0.020	0.08	0.0
	0.101	0.41	0.0
	0.501	2.03	0.0
	2.51	10.2	0.0
	10.0	40.4	0.0
	49.9	202	2.0
	200	808	0.0
Mouse	0.020	0.08	0.7
	0.101	0.41	3.6

Report Title	Study Type	Test Article	Report Number
Protein Binding of 524W91 in Human, Monkey, Mouse, and Rabbit Plasma	Plasma Protein Binding	FTC	TBZZ/93/0025

Study system: In vitro

Target Entity, Test System, and Methods: Plasma, Equilibrium dialysis

Species	FTC concentration		% Bound
	µg/mL	µM	
	0.501	2.03	0.0
	2.51	10.2	2.3
	10.0	40.4	0.0
	49.9	202	3.0
	200	808	3.4
Rabbit	0.020	0.08	2.3
	0.101	0.41	0.0
	0.501	2.03	1.3
	2.51	10.2	0.0
	10.0	40.4	0.0
	49.9	202	0.0
	200	808	0.4

524W91 = emtricitabine

6.3. TAF and TFV

6.3.1. AD-120-2026: Plasma Protein Binding of TAF In Vitro

Report Title:	**Study Type**	**Test Article**	**Report Number**
Plasma Protein Binding of GS-7340	Plasma Protein Binding, In Vitro	TAF	AD-120-2026

Study System: Plasma from Dog and Human

Target Entry, Test System, & Methods: Equilibrium dialysis for 3 hours at 37°C against 0.133 M phosphate buffer, pH 7.4. Analysis by LC-MS/MS.

Matrix	**Fraction Unbound (%)**
Dog plasma	48.0 ± 6.2
Human plasma	46.8 ± 2.3

TAF = tenofovir alafenamide
Note: Incubation concentration of TAF was 2 µM

6.3.2. P0504-00039.1: Protein Binding of TFV

Report Title:	Study Type	Test Article	Report Number
Protein Binding of Cidofovir, Cyclic HPMPC, PMEA, and PMPA in Human Plasma and Serum	Plasma Protein Binding, In Vitro	TFV	P0504-00039.1

Study System: In vitro

Target Entity, Test System, & Method: Plasma/serum, ultrafiltration

Matrix	TFV Concentration	% Unbound
Human Plasma	0.01 µg/mL	96.9
	2.01 µg/mL	99.9
	5.01 µg/mL	101.0
	10.01 µg/mL	95.0
	25.01 µg/mL	103.5
		99.3 (3.3) Mean (SD)
Human Serum	0.01 µg/mL	90.6
	2.01 µg/mL	92.4
	5.01 µg/mL	97.7
	10.01 µg/mL	88.5
	25.01 µg/mL	94.7
		92.8 (3.6) Mean (SD)

7. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS

7.1. BIC

7.1.1. Rats (m2.6.7, Section 14.1, TX-141-2045)

BIC plasma exposure (C_{max} and AUC) increased with the increase in maternal dose level from 2 to 300 mg/kg/day for maternal rats and pups. Sex-based differences were less than 2-fold in BIC C_{max} and AUC_{0-24} values for pups. No accumulation of BIC was observed after repeat dosing in maternal rats. BIC plasma exposure in maternal rats was roughly similar to pups at the 2 mg/kg/day dose level, slightly higher (approximately 1.5-fold) in maternal rats than in pups at the 10 mg/kg/day dose level, and greater than 2-fold higher (approximately 2.8-fold) in maternal rats than in pups at the 300 mg/kg/day dose level.

7.1.2. Rabbits (m2.6.7, Section 11.1, TX-141-2038)

The BIC plasma exposure (C_{max} and AUC) increased with the increase in dose level from 100 to 1000 mg/kg/day in rabbits on Day of Gestation 7 & 19. In general, no accumulation of BIC (< 2.3-fold) was observed after repeat dosing in rabbits.

7.2. FTC

7.2.1. TOX103: Toxicokinetic Study to Determine Fetal Exposure of FTC in Mice

Report Title	Study Type	Test Article	Report Number
Toxicokinetic Study to Determine Fetal Exposures in CD-1 Mice Given TP-0006 Orally	Repeat-Dose Tissue Distribution	FTC	TOX103 Report Addendum

Methods:

Species	Mouse / CD-1
Gestation Day	Gestation days 6-15
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Dose (mg/kg)	1000 (plus 500 only on GD 15)
Analyte	FTC
Assay	LC-MS (SIM)

Plasma:

The mean ± standard deviation for plasma concentrations:

Pregnant mice 137.1 ± 28.0 µg/mL

Pooled fetal homogenate 57.7 ± 10.4 µg/mL

The mean fetal/maternal concentration ratio was 0.41 ± 0.04 µg/mL

Additional Information: One female in the low dose group was not pregnant and was not included in the mean values.

7.2.2. TOX038: Effects of FTC on Embryo/Fetal Development in Rabbits – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
A Study of the Effects of TP-0006 on Embryo/Fetal Development in Rabbits	Repeat-Dose Tissue Toxicokinetics in Embryo/Fetus	FTC	TOX038 Report Addendum

Methods:

Species	Rabbit / New Zealand White
Gestation Day/Number of Animals	Treated on gestation day 7-19 / 20 females per group
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral
Dose (mg/kg)	0, 100, 300, 1000
Analyte	FTC
Assay	LC-MS/MS

Toxicokinetics

Emtricitabine was rapidly absorbed with C_{max} occurring generally within 1 hour postdose. AUC and C_{max} increased linearly with dose. Plasma elimination t½ was 3–4 hours at all dose levels. Fetal/maternal exposure ratios were around 0.4–0.5 one hour after dosing (at t_{max}) for all dose levels. Emtricitabine is readily transferred across the placenta.

Dose (mg/kg/day)	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-12} (µg•h/mL)	AUC_{0-24} (µg•h/mL)	Fetal/Maternal Ratio
100	16.0	1.0	43.6	87.3	0.42
300	44.2	1.4	157.6	315.2	0.51
1000	143.3	1.7	628.9	1257.8	0.41

Additional Information: A NOEL of 100 mg/kg/day was established for maternal toxicity.

AUC_{0-12} = area under the plasma concentration-time curve from zero to 12 hr; AUC_{0-24} = area under the plasma concentration-time curve from zero to 24 hr; C_{max} = maximum plasma concentration; FTC = emtricitabine; LC-MS/MS = liquid chromatography-tandem mass spectrometry; T_{max} = time to reach the maximum plasma concentration

7.3. TAF and TFV

7.3.1. TAF

Studies of TAF in pregnant animals are presented in m2.6.7, Sections 11.3 and 12.4.

7.3.2. TFV

7.3.2.1. 96-DDM-1278-005: Placental Transfer and Pharmacokinetics of TFV in Infant Rhesus Monkeys

Report Title:	**Study Type**	**Test Article**	**Report Number**
Placental Transfer and Pharmacokinetics of PMPA (GS-1278) in Infant Rhesus Monkeys	Placental Transfer	TFV	96-DDM-1278-005

Species	Monkey
Gestation Day / Number of Animals	Daily dosing beginning at gestational day 111, one animal
Vehicle/Formulation	Aqueous suspension
Method of Administration	SC
Dose (mg/kg)/ day	30
Analyte	TFV
Assay	HPLC

	Serum Concentration TFV (µg/mL) 30 mins After Administration					
Time (gestational day)	**115**	**127**	**134**	**140**	**151**	**Mean (SD) [CV%]**
Fetal	7.9	9.1	10.1	15	5.9	9.6 (3.4) [35.4]
Maternal	45.6	61.2	69.4	53.7	56.3	57.2 (8.84) [15.4]
Fetal/Maternal Ratio	0.17	0.15	0.15	0.28	0.11	0.17 (0.07) [38.6]

Additional Information: Based upon the data above it was concluded that placental transfer of TFV appeared to be significant.

7.3.2.2. P2000116: Pharmacokinetics of TFV in Lactating Rhesus Monkeys

Report Title:	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of Tenofovir in Healthy Adult Female Lactating Rhesus Monkeys Following a Single 30 mg/kg Subcutaneous Dose of Tenofovir	Lactating Animals	TFV	P2000116

Species: Monkey

Gestation Day / Number of Animals: Healthy adult female lactating animals

Method of Administration: SC

Dose (mg/kg/day): 30 single dose

Analyte: TFV

Assay: LC-MS/MS

	Animal 1		**Animal 2**	
PK Parameters (n = 2)	**Milk**	**Serum**	**Milk**	**Serum**
C_{max} (µg/mL)	0.808	18.3	0.610	30.2
T_{max} (h)	4	0.5	1	0.5
AUC_{inf} (µg•h/mL)	12.8	68.9	12.1	56.2
AUC Extrapolated (%)	21.7	0.219	23.3	0.193
C_{last} (µg/mL)	0.188	0.0264	0.179	0.0264
T_{last} (h)	24	24	24	24
CL/F (mL/h/kg)	2338	435	2482	534
$MRT_{0-\infty}$ (h)	16.1	2.79	17.0	3.14
$t_{1/2}$ (h)	10.3	3.97	10.9	2.85
V_z/F (mL/kg)	34740	2489	39133	2191

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{last} = last observed quantifiable concentration of the drug in plasma; C_{max} = maximum plasma concentration; F = bioavailability; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MRT = mean residence time; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{last} = time (observed time point) of C_{last}; T_{max} = time to reach the maximum plasma concentration; TFV = tenofovir; V_Z = apparent volume of distribution during the terminal phase

8. PHARMACOKINETICS: METABOLISM IN VIVO

8.1. BIC

8.1.1. AD-141-2304: Metabolite Profiling of Samples from Mice after Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, and Feces Samples from Transgenic Mice after Oral Administration of ^{14}C-GS-9883	Metabolism	[^{14}C]BIC	AD-141-2304

Study System	Metabolite profiling of [^{14}C]BIC in plasma, urine, and feces from Ras H2 mouse [CBYB6Fl-Tg (HRAS) 2Jic] following a 2 mg/kg oral dose	
	Plasma Profile (0-48 h pool)	
Component[a]	**AUC_{0-48h} (ng eq·h/g)**	**% ^{14}C in Plasma AUC pool**
M12	1957	1.86
BIC	100424	95.5
M28	670	0.64
Total in Plasma	105211	100
	Urine Profile (0-24 h pool)	
Component[a]	**% Administered ^{14}C Dose**	
BIC	0.089	
M15	0.88	
M21	0.50	
Other	15 components detected, each < 0.5%	
Total Radioactive Dose in Urine	3.21	

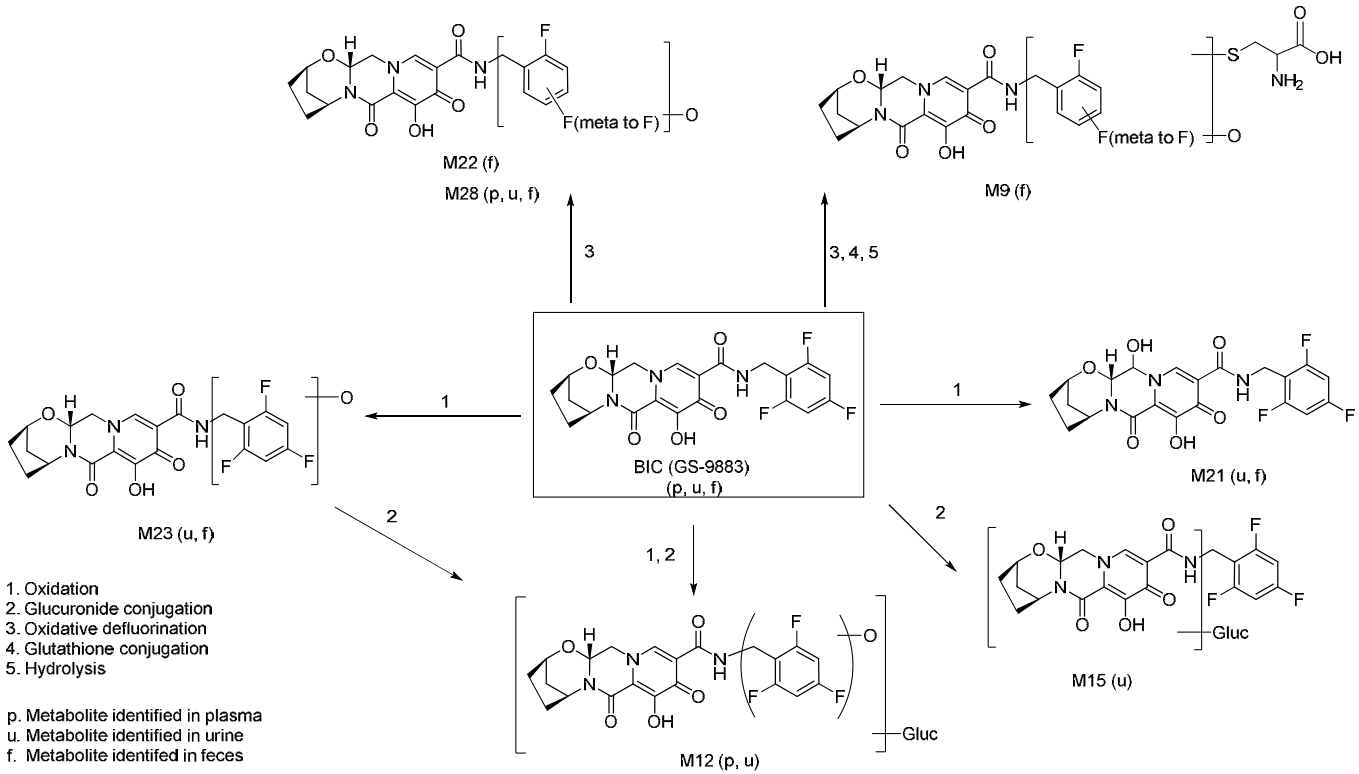
Report Title	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, and Feces Samples from Transgenic Mice after Oral Administration of ^{14}C-GS-9883	Metabolism	[^{14}C]BIC	AD-141-2304

Component[a]	**Feces Profile (0-48 h pool)**
	% Administered ^{14}C Dose
M9	2.38
M21/M22	4.21
M23	3.33
BIC	64.4
M28	4.20
Other	Each component < LOQ
Total Radioactive Dose in Feces	96.7

BIC = bictegravir (GS-9883); LOQ = limit of quantitation (1% of run and 10 cpm peak height); M9 = desfluoro-hydroxy-BIC-cysteine conjugate-2; M12 = hydroxy-BIC-glucuronide; M15 = BIC-glucuronide; M21 = hydroxy-BIC-2; M22 = desfluoro-hydroxy-BIC-2; M23 = hydroxy-BIC-3; M28 = desfluoro-hydroxy-BIC-4; other metabolites were not identifiable due to low concentration

a Proposed structures are shown in Section 8.1.2.

8.1.2. AD-141-2304: Proposed Biotransformation Pathways of [^{14}C]BIC in Mice



BIC = bictegravir

8.1.3. AD-141-2277: Metabolite Profiling of Samples from Rats after Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of ^{14}C-GS-9883	Metabolism	[^{14}C]BIC	AD-141-2277

Study System	Metabolite profiling of [^{14}C]BIC in plasma, urine, bile, and feces from bile duct-intact and bile duct-cannulated male Wistar Han rats following a 2 mg/kg oral dose	
	Plasma Profile (0-168 h pool)	
Component[a]	**$AUC_{0\text{-}168h}$ (ng eq·h/g)**	**% ^{14}C in Plasma AUC pool**
M12	19432	2.18
M20	100279	11.3
M21/M22	10518	1.18
M23	21036	2.36
M26	12657	1.42
BIC	682164	76.5
M28	8379	0.94
Total in Plasma	854465	95.9
	Urine Profile from Bile Duct-Intact Rats (0-48 h pool)	**Urine Profile from Bile Duct-Cannulated Rats (0-48 h pool)**
Component[a]	**% Administered ^{14}C Dose**	**% Administered ^{14}C Dose**
M21	1.01	1.10
M23	0.25	0.81
BIC	0.10	0.068
Other	11 components detected, each < 0.5%	11 components detected, each < 0.5%
Total Radioactive Dose in Urine	3.16	4.64

Report Title Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of ^{14}C-GS-9883	**Study Type** Metabolism	**Test Article** [^{14}C]BIC	**Report Number** AD-141-2277

Component[a]	**Bile Profile (0-168 h pool)** **% Administered ^{14}C Dose**
M1	0.63
M6	0.58
M9	1.16
M10	4.40
M11	0.83
M12	0.55
M13	0.81
M14	0.81
M15	12.7
M16	1.47
M18/M19	3.55
M20	0.54
M21	0.77
BIC	0.58
Other	6 components detected, each < 0.5%
Total Radioactive Dose in Bile	34.1

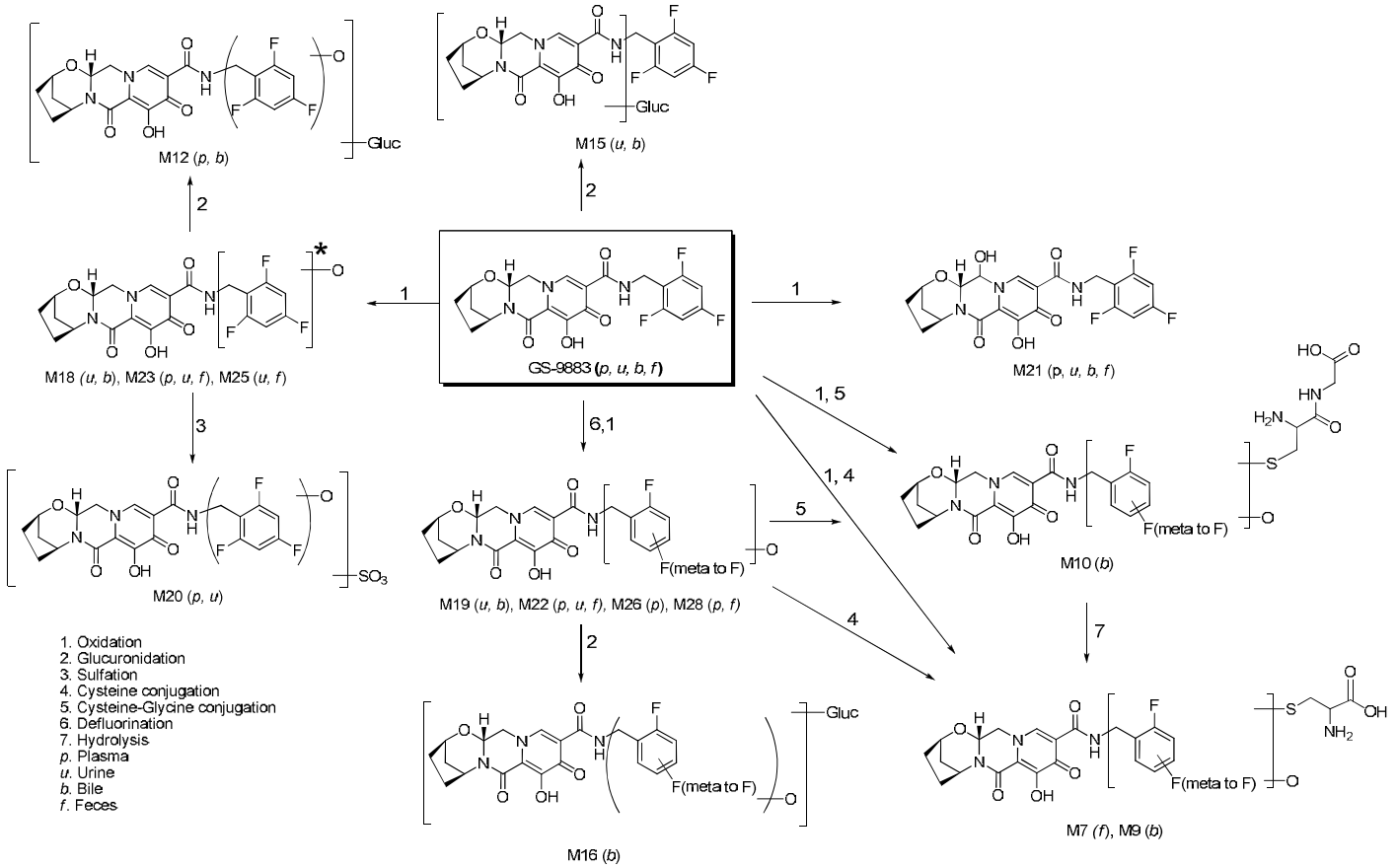
Report Title	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of ^{14}C-GS-9883	Metabolism	[^{14}C]BIC	AD-141-2277

Component[a]	**Feces Profile from Bile Duct-Intact Rats (0-168 h pool)** % Administered Dose	**Feces Profile from Bile Duct-Cannulated Rats (0-168 h pool)** % Administered Dose
M2	0.72	0.41
M7	1.48	0.068
M15	0.74	ND
M16	0.64	0.19
M21/M22	8.07	1.32
M23	3.94	0.81
M25	0.78	0.18
BIC	23.9	13.4
M28	2.08	0.36
Other	5 components detected, each < 0.5%	5 components detected, each < 0.5%
Total Radioactive Dose in Feces	76.4	42.4

BIC = bictegravir (GS-9883); M7 = desfluoro-hydroxy-BIC-cysteine conjugate-1; M9 = desfluoro-hydroxy-BIC-cysteine conjugate-2; M10 = desfluoro-hydroxy-BIC-cysteine-glycine conjugate; M12 = hydroxy-BIC-glucuronide; M15 = BIC-glucuronide; M16 = desfluoro-hydroxy-BIC-glucuronide; M18 = hydroxy-BIC-1; M19 = desfluoro-hydroxy-BIC-1; M20 = hydroxy-BIC-sulfate; M21 = hydroxy-BIC-2; M22 = desfluoro-hydroxy-BIC-2; M23 = hydroxy-BIC-3; M25 = hydroxy-BIC-4; M26 = desfluoro-hydroxy-BIC-3; M28 = desfluoro-hydroxy-BIC-4; other metabolites were not identifiable due to low concentration; ND = peak not detected or below the established limit of quantitation (1% of run and 10 cpm peak height)

a Proposed structures are shown in Section 8.1.4.

8.1.4. AD-141-2277: Proposed Biotransformation Pathways of [^{14}C]BIC in Rats



* Only two structural isomers are possible. However, because we observed three peaks (M18, M23, and M25) with similar mass and fragmentation pattern, we believe the other structure involves a l,2 shift of the fluorine atom during oxidation (Koerts, et. al., 1998).

BIC = bictegravir

8.1.5. AD-141-2299: Metabolite Profiling of Samples from Monkeys after Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Monkeys after Oral Administration of ^{14}C-GS-9883	Metabolism	[^{14}C]BIC	AD-141-2299

Study System	Metabolite profiling of [^{14}C]BIC in plasma, urine, bile, and feces from bile duct-intact and bile duct-cannulated male cynomolgus monkeys following a 1 mg/kg oral dose	
	Plasma Profile (0-72 h pool)	
Component[a]	**AUC_{0-72h} (ng eq·h/g)**	**% ^{14}C in Plasma AUC pool**
M15	196	0.55
M20	277	0.77
M26	312	0.87
M35	468	1.31
M38	450	1.26
M42	4360	12.2
BIC	28700	80.2
Total in Plasma	35800	100
	Urine Profile from Bile Duct-Intact Monkeys (0-48 h pool)	**Urine Profile from Bile Duct-Cannulated Monkeys (0-48 h pool)**
Component[a]	**% Administered Dose**	**% Administered Dose**
M35	3.40	1.45
M15	3.84	3.94
M20/M21/M22	1.90	2.22
M42	6.15	2.61
Other	8 components detected, each < 0.5%	8 components detected, each < 0.5%
Total Radioactive Dose in Urine	19.7	14.6

Report Title Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Monkeys after Oral Administration of ^{14}C-GS-9883	**Study Type** Metabolism	**Test Article** [^{14}C]BIC	**Report Number** AD-141-2299

Component[a]	**Bile Profile (0-48 h pool)** **% Administered Dose**
M30	1.18
M32	2.35
M9	10.9
M35	0.94
M37/M38	1.63
M39	2.45
M15	6.14
M20/M21/M22	8.09
M42	1.16
BIC	0.46
Other	2 components detected, each < 0.5%
Total Radioactive Dose in in Bile	39.4

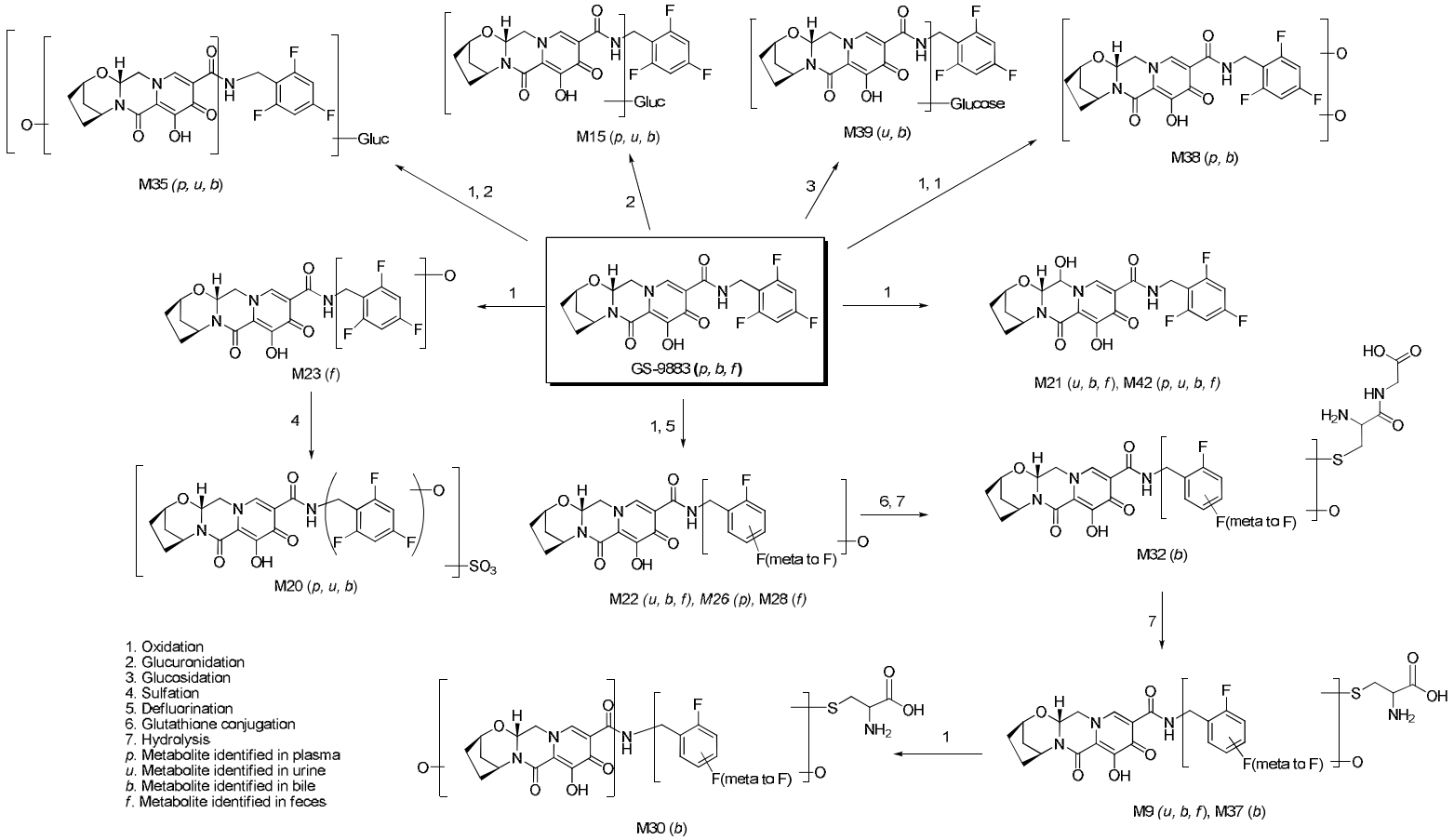
Report Title	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Monkeys after Oral Administration of ^{14}C-GS-9883	Metabolism	[^{14}C]BIC	AD-141-2299

	Feces Profile from Bile Duct-Intact Monkeys (0-96 h pool)	**Feces Profile from Bile Duct-Cannulated Monkeys (0-72 h pool)**
Component[a]	**% Administered Dose**	**% Administered Dose**
M15	0.54	ND
M40	0.93	ND
M21/M22	9.05	3.02
M23/M42	10.5	2.47
BIC	10.7	10.7
Other	2 components detected, each < 0.5%	2 components detected, each < 0.5%
Total Radioactive Dose in Feces	40.0	19.5

BIC = bictegravir (GS-9883); M9 = desfluoro-hydroxy-BIC-cysteine conjugate-2; M15 = BIC-glucuronide; M20 = hydroxy-BIC-sulfate; M21 = hydroxy-BIC-2; M22 = desfluoro-hydroxy-BIC-2; M23 = hydroxy-BIC-3; M26 = desfluoro-hydroxy-BIC-3; M28 = desfluoro-hydroxy-BIC-4; M30 = desfluoro-dihydroxy-BIC-cysteine conjugate; M32 = desfluoro-hydroxy-BIC-cysteine-glycine conjugate-2; M35 = hydroxy-BIC-glucuronide-2; M37 = desfluoro-hydroxy-BIC-cysteine conjugate-3; M38 = dihydroxy-BIC; M39 = BIC-glucoside; M42 = hydroxy-BIC-5; other metabolites were not identifiable due to low concentration; ND = peak below the limit of quantitation

a Proposed structures are shown in Section 8.1.6.

8.1.6. AD-141-2299: Proposed Biotransformation Pathways of [^{14}C]BIC in Monkeys



BIC = bictegravir

8.2. FTC

8.2.1. TEIN/93/0015: Disposition Study of [^{3}H]FTC in Mice

Report Title	Study Type	Test Article	Report Number
Metabolic Disposition and Balance Studies in Male CD-1 Mice Following Oral Administration of 120 mg/kg [6-^{3}H]524W91	Metabolism, Excretion	FTC	TEIN/93/0015

Species	Mouse, CD-1
Sex (M/F)/Number of Animals	15 M,
Feeding Condition	Not fasted
Vehicle/Formulation	Solution in Water
Method of Administration	Oral
Dose (mg/kg)	120 (single dose)
Radionucleotide	^{3}H
Specific Activity	0.65 mCi/mmol

Metabolic Data: Cumulative (0–72 hour postdose) recovery of emtricitabine and its tentatively identified metabolites from the urine of male CD1 mice dosed orally.

Sample	Sampling Time or Period	% of Compound in Sample (Mean ± SD)					
		5-Fluorocytosine	M1/M2[a]	M1/M2[a]	FTC	M3	Unidentified
Urine	0–72 hours	1.4 ± 0.2	1.7 ± 0.3	2.0 ± 0.4	64 ± 7.1	0.5 ± 0.4	0.8 ± 0.2

a Absolute configuration of sulfoxide diastereomers not determined

Report Title	Study Type	Test Article	Report Number
Metabolic Disposition and Balance Studies in Male CD-1 Mice Following Oral Administration of 120 mg/kg [6-^{3}H]524W91	Metabolism, Excretion	FTC	TEIN/93/0015

Excretion Data: The recovery of radioactivity in the urine and feces of male CD1 mice dosed orally.

Sample	Sampling period (hours)	Mean ± SD	
		Percent of dose in sample	Total recovery (%; 0-72 hours)
Urine	0–24	62.3 ± 7.6	66.8 ± 7.0
	24–48	3.4 ± 2.1	
	48–72	1.5 ± 0.4	
Feces	0–24	15.9 ± 3.0	18.1 ± 3.1
	24–48	0.7 ± 0.4	
	48–72	1.5 ± 0.6	
Feces and Urine	0–72	--	85.0 ± 4.2

Additional Information:
Analytical method: Liquid scintillation counting, HPLC radiochromatogram.
Mean ± SD fraction of dose excreted in urine (0–72 hours) as parent drug = 64 ± 7%

8.2.2. TOX063: Metabolism and Excretion of [^{14}C]FTC in Cynomolgus Monkeys

Report Title	Study Type	Test Article	Report Number
Metabolism and excretion of [^{14}C]TP-0006 following oral administration to male Cynomolgus monkeys	Metabolism, Excretion	FTC	TOX063

Species	Monkey, Cynomolgus
Sex (M/F)/Number of Animals	4 M
Feeding Condition	Fasted
Vehicle/Formulation	Sterile water
Method of Administration	Oral
Dose (mg/kg)	200 (single dose)
Radionucleotide	^{14}C
Specific Activity	46 mCi/mmol

Metabolic Data:

Plasma	**FTC**	**M1/M2**
C_{max} (µg/mL)	46.7	15.9
T_{max}(h)	1	2
$AUC_{0\text{-}last}$ (µg•h/mL)	129	56.6
AUC_{inf} (µg•h/mL)	133	86.6
CL/F (L/h/kg)	1.57	-

Excretion Data: 40.8% of the administered radioactivity was recovered in the urine, 35.3% in the feces, and 8.3% in cage washes/wipes. Unchanged parent drug represented the great majority of radioactivity present in urine (approximately 74%) and feces (97%). The recovery of large amounts of radioactivity in the gut contents following a second dose of radioactivity indicates that much of the fecal recovery represented unabsorbed rather than excreted drug.

Distribution Data: 22 tissues obtained 1 hour postdose. Highest levels: kidneys (596 equivalents µg/g); liver (121 µg/ equivalents /g.); CSF/blood ratio 0.031.

8.2.3. TEIN/93/0016: Metabolism and Excretion of [^{3}H]FTC in Cynomolgus Monkeys

Report Title	**Study Type**	**Test Article**	**Report Number**
Metabolic Disposition of 80 mg/kg Orally Administered [6-3H]524W91 in Cynomolgus Monkeys	Metabolism, Excretion	FTC	TEIN/93/0016

Species	Monkey, cynomolgus
Sex (M/F)/Number of Animals	4 F
Feeding Condition	Fasted overnight and to 2 hours postdose
Vehicle/Formulation	Solution in Water
Method of Administration	Oral
Dose (mg/kg)	80 (single dose)
Radionucleotide	^{3}H
Specific Activity	1.8 μCi/mg

Metabolic Data: Cumulative (0–72 hour postdose) urinary and fecal recovery of FTC (% dose).and its tentatively identified metabolites

	Percent of Dose (Mean ± Standard Deviation)								
Sample	**M200**	**5-Fluorocytosine**	**M1/M2**	**M1/M2**	**M1100**	**M3**	**FTC**	**M1940**	**Deaminated FTC**
Urine	0.2 ± 0.1	0.3 ± 0.04	11 ± 4	1.2 ± 0.2	0.3 ± 0.2	1.6 ± 1.9	28.3 ± 4.1	0.1 ± 0.1	1.1 ± 0.3
Feces	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.03 ± 0.1	--	--	34.5 ± 10.7	--	0.5 ± 0.1

Excretion Data: Examined (pool) samples - the recovery of radioactivity in the urine, feces, and cage washes

		Mean ± Standard Deviation	
	Sampling period (hours)	**Percent of dose in sample**	**Total recovery (% ; 0–72 hours)**
Urine	0–8	32.9 ± 8.6	41.2 ± 6.4
	8–24	5.5 ± 1.1	
	24–48	2.2 ± 1.7	
	48–72	0.6 ± 0.9	
Feces	0–24	23.6 ± 15.9	33.1 ± 10.0
	24–48	4.7 ± 5.7	
	48–72	4.8 ± 9.1	
Cage Wash	0–8	3.0 ± 1.9	9.6 ± 6.7
	8–24	5.0 ± 4.0	
	24–48	0.8 ± 0.6	
	48–72	0.8 ± 0.7	
Overall Recovery	0–72	—	83.8 ± 3.8

8.3. TAF

8.3.1. AD-120-2012: Metabolism of TAF in Mouse

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Species: CD-1 mice
Sex (M/F) / No. of Animals: Male/30
Method of Administration: Oral gavage
Dose (mg/kg): 100
Feeding Condition: Not fasted
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample: Plasma
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

Final Metabolite Designation	Concentrations (ng Equivalents [^{14}C]GS-7340/g) 0.25 h	1 h	2 h	12 h	24 h	AUC_{0-24h} (ng eq•h/g)	% AUC of Total Radioactivity
M27A (Allantoin)	549	420	1820	143	121	10530	12.2
M27B (Uric acid)	259	1150	1420	390	371	16670	19.4
M5	1050	239	277	73.4	ND	2411	2.80
M6	280	86.4	160	ND	ND	NA	NA
M28	1690	425	169	ND	ND	878	1.02
M12	13600	4740	5030	1340	385	47167	54.8
M45	2260	667	354	ND	ND	3177	3.69
M46	2740	688	326	ND	ND	2738	3.18
M18	ND	ND	201	ND	ND	NA	NA
M47	ND	ND	166	ND	ND	NA	NA
M19	332	20.9	765	ND	ND	1533	1.78
M23	ND	ND	125	ND	ND	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Species: CD-1 mice

Sex (M/F) / No. of Animals: Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Urine (% Radioactive Dose)				Feces (% Radioactive Dose)			
	Collection Interval (h)				Collection Interval (h)			
Final Metabolite Designation	0-24	24-48	48-72	Total	0-24	24-48	48-72	Total
M27A (Allantoin)	1.56	0.70	0.32	2.57	0.42	ND	0.02	0.43
M5	1.91	0.23	0.02	2.16	NA	NA	NA	NA
M12	13.0	4.12	0.99	18.1	25.3	4.14	1.26	30.7
M28	NA	NA	NA	NA	0.67	ND	ND	0.67
M32	0.36	ND	ND	0.36	NA	NA	NA	NA
M35	0.66	ND	ND	0.66	NA	NA	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Species: CD-1 mice

Sex (M/F) / No. of Animals: Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Kidney Concentration (ng Equivalents [^{14}C]GS-7340/g)			Liver Concentration (ng Equivalents [^{14}C]GS-7340/g)		
	Collection Time (h)			Collection Time (h)		
Final Metabolite Designation	**1**	**24**	**48**	**1**	**24**	**48**
M27A (Allantoin)	NA	NA	NA	107000	63800	35900
M27B (Uric acid)	4250	3050	2180	NA	NA	NA
M7 (Xanthine)	2830	2070	2460	NA	NA	NA
M8 (Hypoxanthine)	1010	731	1190	NA	NA	NA
M12	90300	18000	4620	356000	93200	18500

NA = not applicable; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Species: CD-1 mice

Sex (M/F) / No. of Animals: Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Concentration (ng Equivalents [^{14}C]GS-7340/g)				
	Collection Time (h)				
Final Metabolite Designation	**1**	**4**	**12**	**24**	**48**
M1	392	214	250	325	399
M2	794	326	364	ND	ND
M5	ND	ND	ND	90.6	ND
M7 (Xanthine)	953	ND	ND	46.8	ND
M8 (Hypoxanthine)	254	121	342	298	373
M29	2060	737	599	254	ND
M30	159	ND	164	213	136
M34	ND	ND	ND	70.1	106

ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Study Systems: [^{14}C]GS-7340 (5 µM) was incubated at 37°C with either pooled plasma or hepatic microsomes (with 1 mMNADPH) from male CD-1 mice for 0 and 60 minutes. The extracted samples were analyzed by HPLC with in-line radioactivity detection to determine the metabolite profiles.

	Plasma		Hepatic Microsomes (1 mg/mL Protein)	
	Percent of Radioactivity Injected (% of Run)		Percent of Radioactivity Injected (% of Run)	
Final Metabolite Designation	0 Minute	60 Minute	0 Minute	60 Minute
[^{14}C]M1	NA	NA	ND	0.77
[^{14}C]M2	ND	43.0	3.00	11.1
[^{14}C]M5	NA	NA	ND	ND
[^{14}C]M6	NA	NA	ND	ND
[^{14}C]M28	ND	46.0	2.24	3.98
[^{14}C]M29	NA	NA	ND	0.72
[^{14}C]M30	NA	NA	ND	0.82
[^{14}C]M31	NA	NA	ND	0.35
[^{14}C]M32	ND	5.70	NA	NA
[^{14}C]M33 ([^{14}C]Adenine)	NA	NA	ND	41.0
[^{14}C]M18	43.6	0.87	1.63	0.89
[^{14}C]M20	28.5	ND	ND	ND
[^{14}C]M36	NA	NA	3.35	1.38
[^{14}C]M37	NA	NA	ND	0.70
[^{14}C]M38	NA	NA	ND	3.08
[^{14}C]M39	NA	NA	ND	1.87
[^{14}C]M40	NA	NA	ND	2.96
[^{14}C]M41	NA	NA	ND	0.41
[^{14}C]M42	NA	NA	ND	0.50
[^{14}C]GS-7340	24.1	ND	84.1	16.3

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

8.3.2. AD-120-2021: Metabolism of TAF in Rat

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Species:	Sprague-Dawley rats
Sex (M/F) / No. of Animals:	Male/15
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Not fasted
Vehicle/Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample:	Plasma
Analyte/Assay:	[^{14}C]TAF / Liquid Scintillation Counter

Final Metabolite Designation	Plasma Concentrations (ng Equivalents [^{14}C]GS-7340/g)						AUC_{0-24h} (ng eq·h/g)	% AUC of Total Radioactivity
	0.25 h	0.5 h	1 h	2 h	12 h	24 h		
M27A (Allantoin)	425	259	42.4	8.55	ND	ND	634	23.2
M28	221	135	15.0	ND	ND	ND	159	5.80
M12	246	264	99.7	58.8	19.4	ND	1153	66.7

ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Species: Sprague-Dawley rats

Sex (M/F) / No. of Animals: Male/3

Method of Administration: Oral gavage

Dose (mg/kg): 5

Feeding Condition: Not fasted

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sample: Urine

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)	Feces (Percent of Radioactive Dose)		
	Collection Interval (h)	Collection Interval (h)		
Final Metabolite Designation	0-24	0-24	24-48	Total
M27A (Allantoin)	0.14	NA	NA	NA
M44	1.41	NA	NA	NA
M28	ND	NA	NA	NA
M12	17.1	59.8	3.56	63.3
M32	0.19	NA	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Species: Bile Duct-Cannulated Sprague-Dawley rats

Sex (M/F) / No. of Animals: Male/3

Method of Administration: Oral gavage

Dose (mg/kg): 5

Feeding Condition: Not fasted

Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)	Bile (Percent of Radioactive Dose)	Feces (Percent of Radioactive Dose)		
	Collection Interval (h)		Collection Interval (h)		
Final Metabolite Designation	0-24	0-4	0-24	24-48	Total
M27A (Allantoin)	0.23	NA	NA	NA	NA
M27B (Uric acid)	NA	0.02	NA	NA	NA
M44	1.38	NA	NA	NA	NA
M28	0.38	1.17	NA	NA	NA
M12	17.1	0.67	49.3	12.4	61.7
M32	0.30	NA	NA	NA	NA

NA = not applicable; TAF = tenofovir alafenamide

8.3.3. AD-120-2008: Metabolism of TAF in Dog

Report Title:	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Species: Beagle dogs
Sex (M/F) / No. of Animals: Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample: Plasma
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

Final Metabolite Designation	**Plasma Concentrations (ng Equivalents [^{14}C]GS-7340/g)**						**AUC_{0-24h} (ng eq•h/g)**	**% AUC of Total Radioactivity**
	0.25 h	**0.5 h**	**2 h**	**4 h**	**12 h**	**24 h**		
M2	ND	45.7	58.5	69.1	ND	30.4	1212	7.61
M3	ND	49.0	85.2	67.3	ND	ND	265	1.67
M5	ND	ND	36.9	ND	ND	ND	36.9	0.23
M9	ND	ND	22.2	20.8	ND	ND	65.2	0.41
M10	ND	ND	23.7	ND	ND	ND	23.7	0.15
M12	2760	2070	1050	541	278	175	10874	68.3
M17	213	132	ND	ND	ND	ND	69.8	0.44
M18	4640	1840	38.4	ND	ND	ND	2799	17.6
M20	92.4	76.3	ND	ND	ND	ND	32.6	0.20
TAF	616	420	ND	ND	ND	ND	207	1.30

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title:	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Species: Beagle dogs
Sex (M/F) / No. of Animals: Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample: Urine
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)				
	Collection Interval (h)				
Final Metabolite Designation	**0-24**	**24-48**	**48-72**	**72-96**	**Total**
M1	ND	ND	ND	ND	ND
M2	0.88	0.28	0.16	0.19	1.49
M4	0.69	0.06	0.03	0.02	0.79
M5	0.47	ND	ND	ND	0.47
M11	0.43	ND	ND	ND	0.43
M12	13.3	5.08	3.43	2.35	24.2
M15	0.22	0.02	ND	ND	0.23
M18	ND	0.12	0.07	0.04	0.23
M23	ND	ND	ND	ND	ND
TAF	1.32	ND	ND	ND	1.32

TAF = tenofovir alafenamide; ND = peak was not detected or below the established limit of quantitation

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Species: Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals: Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample: Urine
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)				
	Collection Interval (h)				
Final Metabolite Designation	**0-24**	**24-48**	**48-72**	**72-96**	**Total**
M1	0.12	0.04	0.04	ND	0.19
M2	1.06	0.35	0.14	0.15	1.70
M4	0.36	0.06	0.05	ND	0.48
M5	0.32	0.06	0.03	0.04	0.44
M11	0.39	0.04	ND	ND	0.42
M12	8.97	3.75	2.41	1.68	16.8
M15	ND	ND	ND	ND	ND
M18	ND	ND	ND	ND	ND
M23	0.24	ND	ND	ND	0.24
TAF	1.30	ND	ND	ND	1.30

TAF = tenofovir alafenamide; ND = peak was not detected or below the established limit of quantitation

Report Title:	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Species: Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals: Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Bile (Percent of Radioactive Dose)			
	Collection Interval (h)			
Final Metabolite Designation	0-2	2-4	4-6	Total
M2	0.08	0.02	0.02	0.12
M4	0.23	0.05	0.04	0.32
M5	0.23	0.10	0.10	0.43
M6	0.07	0.02	0.01	0.10
M11	0.04	0.02	0.03	0.09
M12	0.42	0.23	0.32	0.96
M14	0.10	0.06	0.06	0.22
M16	2.72	0.84	0.82	4.38
M17	0.29	0.09	0.10	0.48
M18	2.07	0.64	0.66	3.37
M19	0.01	0.04	0.04	0.09
M21	0.14	0.13	0.11	0.38
M22	0.07	0.09	0.07	0.23
M23	0.02	0.15	0.08	0.24
TAF	0.06	0.09	0.05	0.19

TAF = tenofovir alafenamide; ND = Peak was not detected or below the established limit of quantitation

Report Title:	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Species:	Beagle dogs
Sex (M/F) / No. of Animals:	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle/Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Feces
Analyte/Assay:	[^{14}C]TAF / Liquid Scintillation Counter

	Intact Dog (Feces, Percent of Radioactive Dose)			**BDC Dog (Feces, Percent of Radioactive Dose)**		
	Collection Interval (h)			**Collection Interval (h)**		
Final Metabolite Designation	**0-24**	**24-48**	**Total**	**0-24**	**24-48**	**Total**
M2	0.21	0.04	0.25	ND	ND	ND
M11	0.35	ND	0.35	0.50	0.20	0.70
M12	18.3	2.49	20.8	16.0	10.4	26.4

TAF = tenofovir alafenamide; ND = Peak not detected or below the established limit of quantitation; BDC = bile duct-cannulated

Report Title: Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	**Study Type** Metabolism	**Test Article** [^{14}C]TAF	**Report Number** AD-120-2008

Species: Beagle dogs
Sex (M/F) / No. of Animals: Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample: Bone
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Single Dose		**Multiple Dose**	
	Bone Concentration (ng Equivalents [^{14}C]GS-7340/g)			
	Collection Time (h)			
Final Metabolite Designation	**2**	**24**	**2**	**24**
M2	ND	ND	52.9	ND
M6	ND	ND	45.1	ND
M12	2190	1070	4000	2820

TAF = tenofovir alafenamide; ND = peak not detected or below the established limit of quantitation

Report Title:	**Study Type**	**Test Article**	**Report Number**
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2008

Species: Beagle dogs
Sex (M/F) / No. of Animals: Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle/Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample: Liver
Analyte/Assay: [^{14}C]TAF / Liquid Scintillation Counter

	Single Dose		**Multiple Dose**	
	Liver Concentration (ng equivalents [^{14}C]GS-7340/g)			
	Collection Time (h)			
Final Metabolite Designation	**2**	**24**	**2**	**24**
M2	3120	1230	4440	2610
M7	3840	1160	ND	3260
M8	7760	2680	11700	7340
M12	58200	43300	68100	49400
M13	ND	336	723	716
M18	1570	1040	556	294

TAF = tenofovir alafenamide; ND = peak not detected or below the established limit of quantitation

8.3.4. P2001025: Intracellular Anabolism of TFV in Rhesus Monkeys

Report Title:	Study Type	Test Article	Report Number
Intracellular Kinetics of ^{14}C-PMPA in Rhesus Monkeys	Metabolism	[^{14}C]TFV	P2001025

Species: Rhesus monkeys
Sex (M/F) / No. of Animals: Male/6
Method of Administration: Subcutaneous
Dose (mg/kg): 15, 30 and 60
Feeding Condition: Non fasted
Vehicle/Formulation: Sterile water
Sample: Plasma, PBMC, RBC and Lymph node
Analyte/Assay: [^{14}C]TFV, [^{14}C]TFV-p and [^{14}C]TFV-pp / Liquid Scintillation Counter

	Concentrations in PBMC's, 60 mg/kg (μM)					
	Animal #M95-296			Animal #J93-310		
Time (h)	[^{14}C]TFV	[^{14}C]TFV-p	[^{14}C]TFV-pp	[^{14}C]TFV	[^{14}C]TFV-p	[^{14}C]TFV-pp
1	8.80	0.392	0.627	13.9	0.359	0.669
3	7.71	0.911	1.76	5.51	0.659	1.21
7	3.48	0.682	1.76	0.938	0.319	1.17
16	1.04	0.517	1.51	1.02	0.291	1.64
24	1.99	1.11	2.68	1.66	1.02	2.83
36	0.486	0.597	1.19	0.301	0.228	0.458
48	1.32	0.973	2.55	0.969	0.650	1.70

	Concentrations in RBC's, 30 mg/kg (μM)		
	Animal #2		
Time (h)	[^{14}C]TFV	[^{14}C]TFV-p	[^{14}C]TFV-pp
1	4.33	0.032	0.059
3	1.84	0.058	0.196
7	0.600	0.124	0.383
16	0.206	0.164	0.550
24	0.306	0.292	1.19
36	0.118	0.262	0.922

Report Title: Intracellular Kinetics of ^{14}C-PMPA in Rhesus Monkeys				**Study Type** Metabolism	**Test Article** [^{14}C]TFV	**Report Number** P2001025
48	0.028		0.103		0.372	
Animal #	**#F95-250**			**#J93-335**		
Time (h)	**24**			**48**		
Analyte	**[^{14}C]TFV**	**[^{14}C]TFV-p**	**[^{14}C]TFV-pp**	**[^{14}C]TFV**	**[^{14}C]TFV-p**	**[^{14}C]TFV-pp**
Lpmph Node	pmol/10^6 cells					
Axial	0.212	0.465	0.088	0.074	0.052	0.054
Inguinal	0.021	0.043	0.026	0.257	0.129	ND
Mesenteric	0.028	0.036	0.024	1.03	0.033	0.025

ND = near or below background detection level; PBMC's = peripheral blood mononuclear cells; PMPA = tenofovir (TFV); RBC's = red blood cells; TFV-p = tenofovir monophosphate; TFV-pp = tenofovir diphosphate

9. PHARMACOKINETICS: METABOLISM IN VITRO

9.1. BIC

9.1.1. AD-141-2289: In Vitro Metabolic Stability of BIC in Hepatic Microsomal Fractions

Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-9883 in Hepatic Microsomal Fractions	Metabolism	[^{3}H]BIC	AD-141-2289

Study System	Hepatic microsomal fraction from rats, dogs, monkeys, and humans
Method	[^{3}H]BIC (1 μM) was incubated for up to 65 min at 37°C in the presence of an NADPH regenerating system and UDPGA. The final concentration of microsomal protein in the incubations was 1.0 mg/mL.

Species	In Vitro $t_{1/2}$ (min)	Predicted Hepatic Clearance (L/h/kg)	Predicted Hepatic Extraction (%)
Sprague-Dawley Rat	49	1.21	29
Beagle Dog	108	0.29	16
Cynomolgus Monkey	63	0.43	27
Rhesus Monkey	76	0.41	18
Human	194	0.17	13

BIC = bictegravir (GS-9883); NADPH = β-nicotinamide adenine dinucleotide phosphate (reduced form); UDPGA = uridine diphosphate glucuronic acid

9.1.2. AD-141-2290: Cytochrome P450 Reaction Phenotyping of BIC

Report Title	Study Type	Test Article	Report Number
Cytochrome P450 Metabolic Reaction Phenotyping of GS-9883	Metabolism	[^{3}H]BIC	AD-141-2290

Study System	Individual cDNA expressed human CYP enzyme preparations (Supersomes^{TM})
Method	[^{3}H]BIC (2 μM) was incubated with individual cDNA expressed human CYP enzyme preparations co-expressed with human NADPH CYP reductase (Supersomes^{TM}) for 45 min. Compounds known to be metabolized by each CYP enzyme were used as positive controls.

	Positive Control Metabolism			BIC Metabolism	
Enzyme	**Substrate**	**Rate of metabolite formation (min^{-1})**	**Loss of substrate**	**% M1[a]**	**% M2[a]**
CYP1A1	Phenacetin	--	6.8%	ND	ND
CYP2B6	Bupropion	0.141	--	ND	ND
CYP2C8	Paclitaxel	0.008	--	ND	ND
CYP2C9	Diclofenac	0.004	--	ND	ND
CYP2C19	S Mephenytoin	0.020	--	ND	ND
CYP2D6	Dextromethorphan	0.014	--	ND	ND
CYP3A4	Terfenadine	0.032	--	39	1.9
CYP3A5	Terfenadine	0.032	--	55	7.0

BIC = bictegravir (GS-9883); CYP = cytochrome P450 enzyme; ND = not detected; M1 = metabolite 1; M2 = metabolite 2; NADPH = β-nicotinamide adenine dinucleotide phosphate (reduced form)
a M1 and M2 are oxidative metabolites formed in presence of NADPH; exact identity was not determined.

9.1.3. AD-141-2291: UDP-Glucuronosyl Transferase Reaction Phenotyping of BIC

Report Title	Study Type	Test Article	Report Number
UDP-Glucuronosyl Transferase Phenotyping of GS-9883	Metabolism	BIC	AD-141-2291

Study System	Individual cDNA expressed human UGT enzyme preparations (Supersomes™)
Method	BIC (5 μM) was incubated with individual cDNA expressed human UGT enzyme preparations (Supersomes™) for 60 min. Compounds known to be metabolized by each UGT enzyme were used as positive controls.

Enzyme	Positive Control	Positive Control (% remaining at 60 min)	BIC Glucuronide Formation (PAR × 10^{-3} at 60 min)
UGT1A1	Raloxifene	26	12.0
UGT1A3	Raloxifene	52	1.0
UGT1A4	Trifluoperazine	69	ND
UGT1A6	7-Hydroxycoumarin	<10	ND
UGT1A7	7-Hydroxycoumarin	33	ND
UGT1A8	7-Hydroxycoumarin	74	1.0
UGT1A9	7-Hydroxycoumarin	<10	3.0
UGT1A10	Raloxifene	12	ND
UGT2B4	4-Hydroxyestradiol	45	ND
UGT2B7	4-Hydroxyestradiol	<10	ND
UGT2B15	Scopoletin	<10	ND
UGT2B17	4-Hydroxyestradiol	42	ND

BIC = bictegravir (GS-9883); ND = not detected; PAR = peak area ratio of analyte to internal standard; UDP = uridine diphosphate; UGT = UDP-glucuronosyltransferase

9.1.4. AD-141-2288: Metabolites of BIC Detected in Cryopreserved Hepatocytes

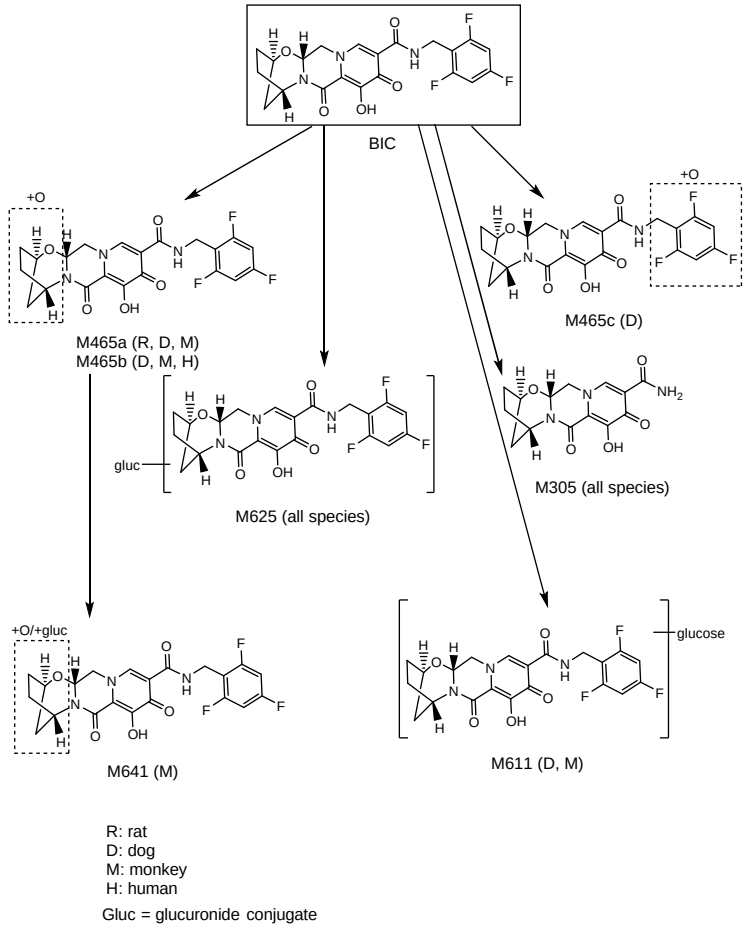
Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of [^{14}C]-GS-9883 in Cryopreserved Hepatocytes from Han-Wistar Rats, Beagle Dogs, Cynomolgus Monkeys and Humans	Metabolism	[^{14}C]BIC	AD-141-2288

Study System	Cryopreserved hepatocytes from rats, dogs, monkeys, and humans
Method	[^{14}C]BIC (20 μM) was incubated in a 24-well plate containing cryopreserved hepatocyte suspensions (1 million cells/mL) from each species at 37ºC for 4 hours.

	Percent Relative Abundance at 4 Hours[a]							
Species	**M305**	**M465a**	**M465b**	**M465c**	**M611**	**M625**	**M641**	**BIC**
Wister Han Rat	1.7	1.2	ND	ND	ND	5.2	ND	91.5
Beagle Dog	8.7	1.4	0.2	3.6	0.8	6.6	ND	78.7
Cynomolgus Monkey	2.4	2.7	11.6	ND	4.4	21.7	4.1	52.4
Human	1.2	ND	0.6	ND	ND	4.3	ND	93.9

BIC = bictegravir (GS-9883, mass =449); M305 = N-dealkylated-BIC; M465a = Hydroxy-BIC-a; M465b = Hydroxy-BIC-b; M465c = Hydroxy-BIC-c; M611 = BIC-glucose; M625 = BIC-glucuronide; M641 = Hydroxy-BIC-glucuronide; ND = not determined
a Determined by comparison of radiochromatographic peak area.

9.1.5. AD-141-2288: Proposed Biotransformation Pathways of [^{14}C]BIC in Cryopreserved Hepatocytes from Rats, Dogs, Monkeys and Humans



BIC = bictegravir (GS-9883)

9.2. FTC

9.2.1. 15396v1: Human Cytochrome P450 Reaction Phenotyping and Glucuronidation Potential of FTC

Report Title	Study Type	Test Article	Report Number
Identification of the Principal Human Cytochrome P450 Isoenzyme(s) and Potential Glucuronidation Responsible for the Metabolism of Emtricitabine (FTC) using Pooled Human Liver Microsomes and Bactosomes Containing cDNA-expressed Human Cytochrome P450 (CYP) Isoenzymes	Metabolism	FTC	15396V1

Study System	Study system 1) cDNA-Expressed Human Cytochromes P450 (CYP1A2, 2A6, 2B6, 2C8, 2C9 2C19, 2D6, 2E1 and 3A4) Study system 2) Pooled Human Hepatic Microsomal Fraction and Enzyme-Selective Inhibitors
Results	• One minor metabolite (~1%) was detected in cDNA-expressed CYP3A4 incubations • No metabolites were formed by CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6 or 2E1 • Microsomal incubations in the presence and absence of selective inhibitors confirmed the low rate of metabolism, and also suggested the possible involvement of flavin-containing monooxygenase enzymes (due to lack of complete inhibition by the CYP3A-selective inhibitor, ketoconazole • No glucuronidation was observed pooled human liver microsomal fraction in the presence of UDPGA
Conclusions	• Emtricitabine was relatively stable in the presence of cytochrome P450 enzymes and is not a substrate for hepatic glucuronidation

9.3. TAF

9.3.1. AD-120-2025: Metabolism of TAF In Vitro (Plasma Stability)

Report Title:	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Plasma from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2025

Study Systems: Duplicate aliquots of 3 µM TAF were incubated with pooled plasma from beagle dog and human at 37°C up to 4 hours. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC-MS/MS.

Species	Half-Life (min)
Dog	69.5 ± 3.20
Human	74.7 ± 6.40

TAF = tenofovir alafenamide

9.3.2. AD-120-2023: Metabolism of TAF In Vitro in Hepatic S9

Report Title:	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Hepatic Subcellular Fractions from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2023

Study Systems: Duplicate aliquots of 3 μM TAF were incubated with pooled hepatic S9 fractions from beagle dog and human at 37°C up to 90 minutes. Rates of metabolism (in vitro half-life values) were determined and hepatic extraction was predicted using the well-stirred liver model. Analysis was done by LC-MS/MS.

Species	Half-Life (min)	Predicted Hepatic Extraction (%)
Dog	31.1 ± 3.40	60.5
Human	20.6 ± 0.70	76.2

TAF = tenofovir alafenamide

9.3.3. AD-120-2024: Metabolism of TAF In Vitro in Intestine S9

Report Title:	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Intestinal Subcellular Fractions from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2024

Study Systems: Duplicate aliquots of 3 µM TAF were incubated with pooled intestinal S9 fractions from beagle dog and human at 37°C for up to 120 minutes. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC-MS/MS.

Species	Half-Life (min)
Dog	47.1 ± 2.60
Human	58.3 ± 4.40

TAF = tenofovir alafenamide

9.3.4. AD-120-2004: Human Cytochrome P450 Metabolic Reaction Phenotyping of TAF

Report Title:	Study Type	Test Article	Report Number
Cytochrome P450 Metabolic Reaction Phenotyping of GS-7340	Metabolism	TAF	AD-120-2004

Methods: Rates of metabolism of TAF (5 µM) catalyzed by cDNA expressed major human cytochrome P450 enzyme preparations co-expressed with NADPH CYP450 reductase

	Metabolism Rate (min^{-1})					
Test Compound	**CYP1A2**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**
GS-7340 (% Positive Control)	< 0.12 (< 0.9%)	0.23 (< 0.8%)	< 0.47 (< 1.0%)	< 0.12 (< 40%)	< 0.23 (< 0.9%)	1.9 (26.6%)
Ethoxycoumarin	13.2	-	-	-	-	-
Amodiaquine	-	29.3	-	-	-	-
Diclofenac	-	-	47.3	-	-	-
Diazepam	-	-	-	0.29[a]	-	-
Dextromethorphan	-	-	-	-	25.4	-
Testosterone	-	-	-	-	-	7.2

TAF = tenofovir alafenamide; NA = not applicable
a Diazepam is a selective substrate for CYP2C19 but is metabolized slowly.

9.3.5. AD-120-2017: Metabolism of TAF In Vitro in Primary Human Hepatocytes

Report Title:	Study Type	Test Article	Report Number
In Vitro Activation of GS-1278, GS-4331 and GS-7340 in Primary Human Hepatocytes	Metabolism, In Vitro	TFV, TDF, TAF	AD-120-2017

Study Systems: Duplicate wells of 5 µM test compounds were incubated with primary human hepatocytes for 24 hours. Intracellular concentrations of TFV-DP were determined. Analysis was done by LC-MS/MS.

Sampling Time: 24 hours

	TFV-DP Concentration (pmol/million cell)
TFV	12.1
TDF	302
TAF	1,470

TAF = tenofovir alafenamide; TDF = tenofovir disoproxil; TFV = tenofovir; TFV-DP = tenofovir diphosphate

9.3.6. 96-DDM-1278-003: Metabolism of TFV in Dog Plasma, Hepatic S9 and Intestinal S9, and Rat Hepatic Microsomal Fraction

Report Title:	**Study Type**	**Test Article**	**Report Number**
In Vitro Metabolism of [^{14}C]PMPA in Human and Animal Tissues	Metabolism, In Vitro	TDF	96-DDM-1278-003

Study Systems: Study system 1: rat hepatic microsomal fraction from control animals or following treatment with the general inducer Aroclor 1254
Study system 2: plasma, intestinal S9 and hepatic S9 from human and dog

	In Vitro Half Life (min) (% TFV Remaining Following 60 min Incubation at 37°C)			
	Liver Microsomes / S9	**Aroclor Hepatic Microsomal Fraction**	**Plasma**	**Intestinal S9**
Rat	> 60 (117%)[a]	> 60 (93%)[a]	-	-
Rat (+ NADPH)	> 60 (111%)[a]	> 60 (99%)[a]	-	-
Dog	> 60 (92%)[b]	-	> 60 (98%)[b]	> 60 (110%)[b]
Human	> 60 (108%)[b]	-	> 60 (111%)[b]	> 60 (116%)[b]

Additional Information: No metabolites were detected.
a Data from study system 1
b Data from study system 2

9.3.7. AD-120-2027: Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on TAF Metabolism In Vitro

Report Title:	**Study Type**	**Test Article**	**Report Number**
Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on the In Vitro Metabolism of GS-7340 in Human Intestinal Subcellular Fraction	Drug-drug interaction (in vitro)	TAF	AD-120-2027

Methods: Duplicate aliquots of 2 µM TAF were incubated with HIV protease inhibitors, atazanavir and darunavir, and the pharmacokinetic enhancers, ritonavir and cobicistat in pooled human intestinal S9 fractions at 37ºC up to 120 minutes. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC-MS/MS.

Inhibitor or Enhancer	**Concentration (µM)**	**TAF Intestinal S9 Stability, $t_{½}$ (min)**
Vehicle Control	0	24.5 ± 4.1
Atazanavir	25	28.9 ± 5.2
	100	38.9 ± 5.3
Darunavir	25	32.2 ± 5.1
	100	30.8 ± 5.0
Ritonavir	25	19.0 ± 2.8
	100	18.9 ± 1.5
Cobicistat	25	30.1 ± 5.1
	100	32.9 ± 4.8
Dichlorvos (Control)	500	> 789[a]

TAF = tenofovir alafenamide
a Less than 10% loss of substrate in 120 min

9.3.8. AD-120-2031: Effect of Inhibitors of CatA, CES1, and CYP3A4 on TAF Metabolism In Vitro

Report Title:	Study Type	Test Article	Report Number
Effect of Inhibitors of Cathepsin A, Carboxylesterase 1, and CYP3A4 on Metabolism of Tenofovir Alafenamide Fumarate (GS-7340) in Primary Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2031

Methods The effect of inhibitors of cathepsin A (CatA), carboxylesterase 1 (CES1), and CYP3A on TAF activation to the pharmacologically active nucleotide analog diphosphate, tenofovir diphosphate (TFV-DP), was assessed in primary human hepatocytes. The duplicate wells were incubated with 0.5 µM TAF in the presence and absence of the inhibitors (concentrations at 0, 0.08, 0.4, 2, 10, and 50 µM). The amount of TFV-DP formation was analyzed by LC-MS/MS.

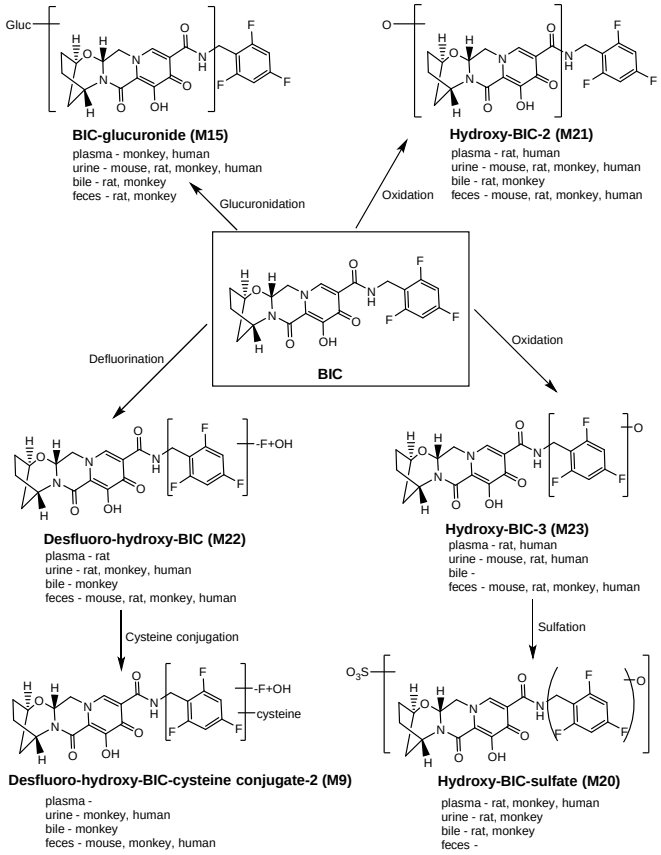
	Intracellular TFV-DP (% Control)				
Inhibitor Concentration (µM)	**Telaprevir**	**Boceprevir**	**BNPP**	**COBI**	**Telaprevir + BNPP**
0	100	100	100	100	100
0.08	109	91.2	124	123	132
0.4	93.8	91.1	94.1	98.3	103
2	103	91.7	63.4	100	51.9
10	106	87.2	70.2	88.2	16.1
50	984	100	33.6	87.9	5.30

CatA = cathepsin A; CES1 = carboxylesterase 1; CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide
Note: Telaprevir and boceprevir are CatA inhibitors; cobicistat is a CYP3A inhibitor; bis(p-nitrophenyl) phosphate (BNPP) is a CES1 inhibitor

10. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

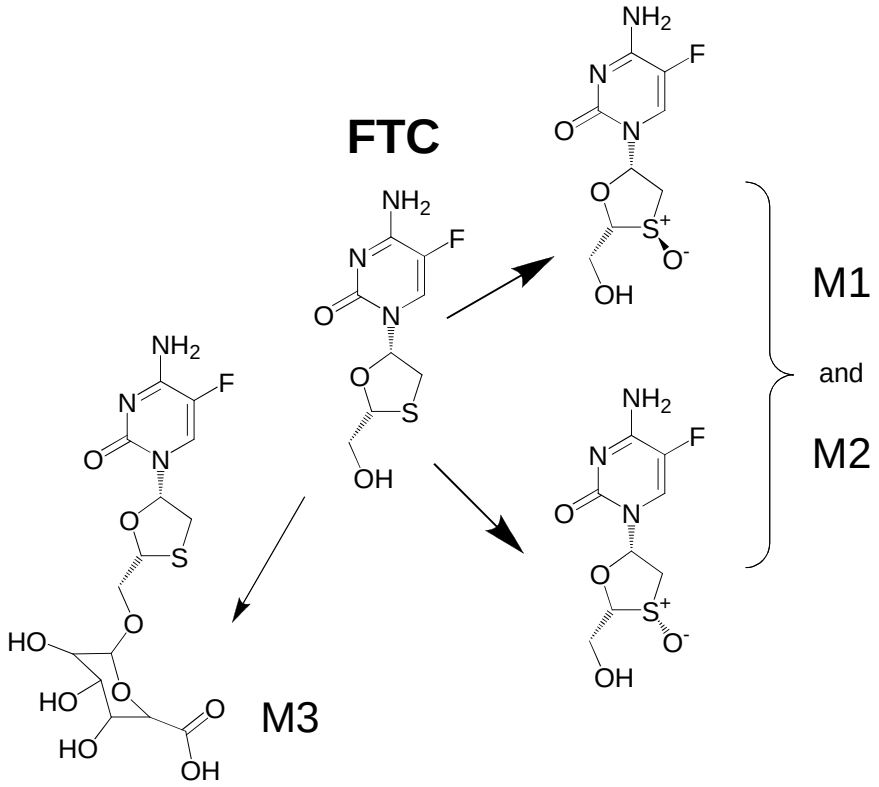
10.1. BIC

10.1.1. Metabolites Identified in Mouse, Rat, Monkey and Human Following a Single Dose of [^{14}C]BIC



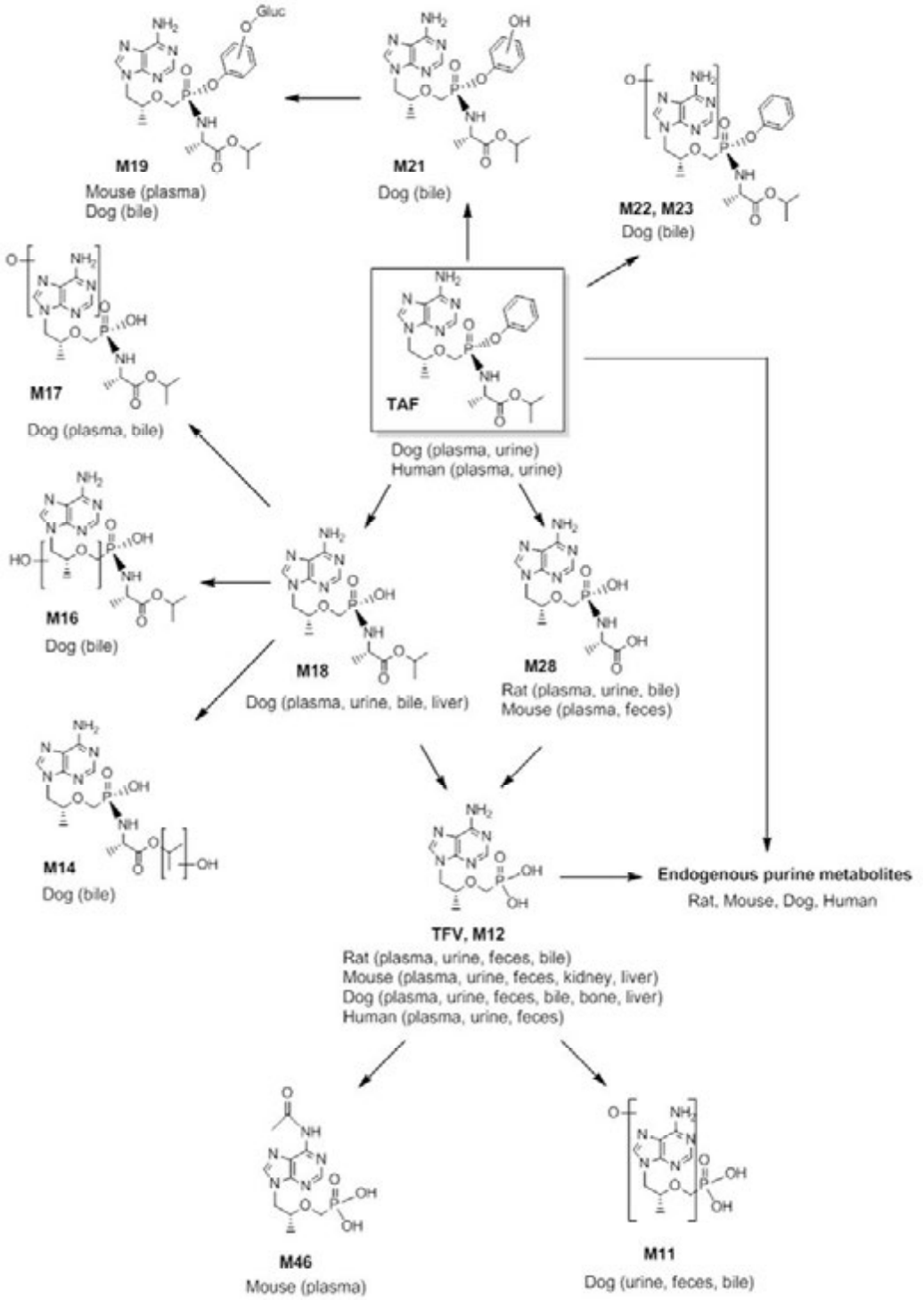
10.2. FTC

The diagram shows the conversion of FTC to its major metabolites: 3'-sulfoxide diastereomers (M1 and M2), and 2'-O-glucuronide (M3).



10.3. TAF

The diagram shows TAF metabolites observed in rats, mice, and dogs after a single oral dose of [^{14}C]TAF.



11. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLIZING ENZYMES

11.1. BIC

11.1.1. AD-141-2293: CYP Inhibition Potential of BIC

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-9883	Metabolism	BIC	AD-141-2293

Study System	Human liver microsomes
Method	BIC (0 – 100 µM) or control inhibitors[a] were incubated with human liver microsomes and NADPH in the presence of probe substrates for individual enzymes. All assays were conducted under conditions that were linear with respect to time and protein concentration. The enzyme-specific probe substrate metabolites were quantified by LC-MS/MS.

		Control Inhibitor	BIC	
CYP Enzyme	**Activity**	**IC_{50} (µM)[b]**	**% inhibition at 100 µM**	**IC_{50} (µM)[b]**
CYP1A2	Phenacetin *O*-deethylase	0.06	-0.987	>100
CYP2B6	Bupropion 4-hydroxylase	1.70	13.3	>100
CYP2C8	Paclitaxel 6α-hydroxylase	1.07	23.5	>100
CYP2C9	Tolbutamide 4-hydroxylase	0.63	40.4	>100
CYP2C19	*S*-Mephenytoin 4'-hydroxylase	10.4	42.0	>100
CYP2D6	Dextromethorphan *O*-demethylase	0.05	0.737	>100
CYP3A	Midazolam 1'-hydroxylase	0.05	34.3	>100
	Testosterone 6β-hydroxylase	0.17	33.8	>100

BIC = bictegravir (GS-9883); CYP = cytochrome P450 enzyme

a Control Inhibitors: CYP1A2, α-Naphthoflavone (0–3 µM); CYP2B6, ticlopidine (0-10 µM); CYP2C8, Montelukast (0–3 µM); CYP2C9, Sulfaphenazole (0–10 µM); CYP2C19, Tranylcypromine (0–50 µM); CYP2D6, Quinidine (0–3 µM); CYP3A, Ketoconazole (0–3 µM).

b Values are the mean of 7 determinations.

11.1.2. AD-141-2294: UGT1A1 Inhibition Potential of BIC

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-9883	Metabolism	BIC	AD-141-2294

Study System	Human microsomal fraction containing human UGT1A1
Method	BIC (0 – 300 µM) or the control inhibitor (atazanavir; ritonavir) was incubated with hepatic microsomal protein (0.3 mg/mL), alamethicin (15 µg/mL), UDP-glucuronic acid (5 mM), and the probe substrate, β-estradiol (17 µM). The UGT1A1-selective metabolite, estradiol 3-glucuronide, was monitored by LC-MS/MS and a decrease in the formation of the metabolite compared to the vehicle control was used to calculate an IC_{50} value.

		Calculated IC_{50} (µM)		
Enzyme	**Activity**	**Atazanavir**	**Ritonavir**	**BIC**
UGT1A1	β-Estradiol-3-glucuronidation	0.33	3.04	176

BIC = bictegravir (GS-9883); UGT = uridine diphosphate glucuronosyl transferase

11.1.3. AD-141-2308: CYP Mechanism-Based Inhibition Potential of BIC

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human Hepatic Microsomal Cytochrome P450 Mechanism-Based Inhibition Potential of GS-9883	Metabolism	BIC	AD-141-2308

Method	The potential for BIC (100 μM) to act as a mechanism based inhibitor of human hepatic microsomal cytochromes P450 drug metabolizing enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A was assessed with a two-stage incubation protocol. The first stage allowed for inactivation of the enzyme in the absence of substrate, and the second stage was used to assay the remaining enzyme activity. A 10-fold dilution was performed between the 2 stages to reduce the direct inhibitory effects of the test compounds. The enzyme-specific metabolites were quantified by LC-MS/MS.

Enzyme	Activity	% Change Over Vehicle Control	
		Control Inhibitor[a]	BIC
CYP1A2	Ethoxyresorufin O-deethylase	71.4 ± 2.0 62.1 ± 2.6	-6.6 ± 5.4
CYP2B6	Bupropion 4-hydroxylase	82.8 ± 2.4	1.6 ± 8.7
CYP2C8	Paclitaxel 6α-hydroxylase	44.4 ± 3.8	6.7 ± 3.8
CYP2C9	Diclofenac 4'-hydroxylase	77.5 ± 5.0	0.6 ± 15.6
CYP2C19	S-Mephenytoin 4’-hydroxylase	55.6 ± 2.2	-1.3 ± 4.6
CYP2D6	Dextromethorphan O-demethylase	82.1 ± 0.8	12.1 ± 7.7
CYP3A	Midazolam 1’-hydroxylase	57.9 ± 2.6 78.5 ± 0.8	39.8 ± 3.7
	Testosterone 6β-hydroxylase	90.4 ± 1.8 66.8 ± 2.2	7.4 ± 9.9

BIC = bictegravir (GS-9883); CYP = cytochrome P450 enzyme
a Control Inhibitors: CYP1A2, resveratrol and furafylline; CYP2B6, ticlopidine; CYP2C8, gemfibrozil glucuronide; CYP2C9, tienilic acid; CYP2C19, ticlopidine; CYP2D6, paroxetine; CYP3A, mibefradil and mifepristone

11.1.4. AD-141-2292: In Vitro Assessment of the Effect of BIC on AhR and PXR

Report Title	Study Type	Test Article	Report Number
Induction Potential of GS-9883 Assessed In Vitro	Metabolism	BIC	AD-141-2292

Study System:	For AhR activation assay, the Dioxin Response Element (DRE) 12.6 cells were transformed with an expression vector for human AhR and the DRE of the human CYP1A2 gene linked to a luciferase reporter. For PXR activation assay, DPX2 cells were stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase.
Method	BIC (0.15 – 50 μM) or positive controls were incubated with the cells for 24 h, after which the luciferase substrate 5'-fluoroluciferin was added and the luminescence was read in a luminometer. The average luminescent units for three replicates were divided by the average for the DMSO solvent control to determine the fold-activation.

	Fold Activation Over 0.1% DMSO Control			
	AhR		PXR	
Concentration (μM)	BIC	β-naphthoflavone[a]	BIC	Rifampicin[a]
0.1	ND	1.09	ND	1.26
0.15	0.72	ND	0.93	ND
0.5	0.49	2.20	0.97	3.02
1.0	ND	3.22	ND	4.45
1.5	0.50	ND	1.03	ND
5.0	0.48	13.9	1.48	8.74
10	ND	23.5	ND	10.4
15	0.63	ND	2.75	ND
20	ND	24.5	ND	10.8
50	0.90	ND	4.22	ND

AhR = aryl hydrocarbon receptor; BIC = bictegravir (GS-9883); CYP = cytochrome P450 enzyme; DMSO = dimethyl sulfoxide; ND = not determined; PXR = pregnane X receptor.
a β-naphthoflavone is a positive control for AhR activation; rifampicin is a positive control for PXR activation.

11.1.5. AD-141-2305: Induction Potential of BIC in Cultured Human Hepatocytes

Report Title	Study Type	Test Article	Report Number
Induction Potential of Bictegravir Assessed in Human Hepatocytes	Metabolism	BIC	AD-141-2305

Method	Human hepatocytes from 3 separate donor livers were incubated with BIC, positive controls (omeprazole, phenobarbital, and rifampin) and solvent vehicle controls, in 3 preparations. The medium was replaced daily with fresh medium containing BIC or the controls. After 3 days of treatment, microsomes were isolated from the harvested cells, incubated with enzyme specific probe substrates and the probe substrate metabolites were quantified by LC-MS/MS. In addition, mRNA levels in the harvested cells were analyzed by qRT-PCR to assess the effect of BIC on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3A4, UGT1A1, and P-gp mRNA expression.

	Fold Increase in CYP Activity over vehicle control (mean % over positive control)		
Treatment	**CYP1A2**	**CYP2B6**	**CYP3A4/5**
BIC (1 μM)	1.00 ± 0.09 (0.00%)	0.95 ± 0.09 (-0.45%)	1.19 ± 0.15 (2.24%)
BIC (3 μM)	1.01 ± 0.11 (0.068%)	1.09 ± 0.16 (0.82%)	1.36 ± 0.26 (4.24%)
BIC (10 μM)	0.86 ± 0.07 (-0.96%)	1.15 ± 0.12 (1.36%)	2.50 ± 0.51 (17.7%)
BIC (30 μM)	0.63 ± 0.07 (-2.53%)	0.76 ± 0.07 (-2.18%)	2.57 ± 0.23 (18.5%)
BIC (60 μM)	0.42 ± 0.06 (-3.97%)	0.73 ± 0.09 (-2.45%)	1.73 ± 0.23 (8.60%)
Omeprazole (50 μM)[a]	15.6 ± 4.3	NA	NA
Phenobarbital (1000 μM)[a]	NA	12.0 ± 0.8	NA
Rifampin (10 μM)[a]	NA	NA	9.49 ± 1.00

Report Title	Study Type	Test Article	Report Number
Induction Potential of Bictegravir Assessed in Human Hepatocytes	Metabolism	BIC	AD-141-2305

Treatment	mRNA Fold Increase Over Vehicle Control (mean % over positive control)						
	CYP1A2	CYP2B6	CYP3A4	CYP2C8	CYP2C9	UGT1A1	P-gp
BIC (1 μM)	1.10 ± 0.22 (0.61%)	1.19 ± 0.41 (1.81%)	1.71 ± 1.55 (3.13%)	1.62 ± 0.16 (67.4%)	1.07 ± 0.28 (36.8%)	1.18 ± 0.45 (1.80%)	1.02 ± 0.51 (2.27%)
BIC (3 μM)	1.11 ± 0.30 (0.67%)	1.63 ± 0.33 (6.00%)	2.50 ± 1.29 (6.61%)	1.67 ± 0.17 (72.8%)	1.18 ± 0.15 (94.7%)	1.22 ± 0.21 (2.20%)	0.87 ± 0.14 (-14.8%)
BIC (10 μM)	1.22 ± 0.23 (1.35%)	2.41 ± 0.75 (13.4%)	7.20 ± 3.15 (27.3%)	1.98 ± 0.38 (107%)	1.26 ± 0.20 (137%)	1.89 ± 0.51 (8.90%)	1.00 ± 0.25 (0.00%)
BIC (30 μM)	1.18 ± 0.29 (1.10%)	3.93 ± 1.14 (27.9%)	16.4 ± 12.7 (67.8%)	1.78 ± 0.41 (84.8%)	1.20 ± 0.26 (105%)	3.55 ± 0.95 (25.5%)	1.38 ± 0.67 (43.2%)
BIC (60 μM)	0.74 ± 0.25 (-1.60%)	4.74 ± 1.64 (35.6%)	16.7 ± 9.1 (69.2%)	1.10 ± 0.24 (10.9%)	1.37 ± 0.38 (195%)	3.51 ± 0.92 (25.1%)	5.76 ± 2.00 (541%)
EC_{50}[b] (μM)	NA	103	19.1	NA	NA	143	NA
Omeprazole (50 μM)[a]	17.3 ± 3.5	NA	NA	NA	NA	11.0 ± 3.6	NA
Phenobarbital (1000 μM)[a]	NA	11.5 ± 5.1	NA	NA	NA	NA	NA
Rifampin (10 μM)[a]	NA	NA	23.7 ± 8.4	1.92 ± 0.62	1.19 ± 0.22	NA	1.88 ± 0.65

BIC = bictegravir; CYP = cytochrome P450 enzyme; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; NA = not applicable; P-gp = P-glycoprotein; UGT = UDP glucuronosyl transferase
Data are the mean ± standard deviation from 3 donors
a Positive control inducers: CYP1A2 - omeprazole; CYP2B6 - phenobarbital; CYP3A4, CYP2C8, CYP2C9, P-gp – rifampin
b Values were extrapolated by curve fitting (constrained to E_{max} of 100%)

11.2. FTC

11.2.1. 15247: Evaluation of FTC as an Inhibitor of Human Cytochromes P450 and Uridine Diphosphate Glucuronosyl Transferase Activity

Report Title	Study Type	Test Article	Report Number
In Vitro Evaluation of Emtricitabine (FTC) as an Inhibitor of Human Cytochrome P450 Enzymes and 5'-Uridine Diphosphate Glucuronosyl Transferase (UGT)	Metabolism	FTC	15247

Methods: Pooled human hepatic microsomal fraction was utilized to measure the activities: 7-ethoxyresorufin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), 7-benzyloxyresorufin *O*-debenzylation (CYP2B6), tolbutamide methyl hydroxylation (CYP2C9), (*S)* mephenytoin 4'-hydroxylation (CYP2C19), dextromethorphan *O*-demethylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation (CYP3A) and 7-hydroxycoumarin glucuronidation (various UGTs), in the presence of various concentrations of FTC. Selective inhibitors were tested to confirm the sensitivity of the assays

Enzyme	Enzyme Reaction	Control Inhibitor	FTC		Control Inhibitor
			K_i (μM)	Type of Inhibition	K_i (μM)
CYP1A2	7-ethoxyresorufin *O*-deethylation	α-naphthoflavone	-	No inhibition	Competitive 0.011
CYP2A6	coumarin 7-hydroxylation	tranylcypromine	-	No inhibition	Mixed: Competitive 1.2/Uncompetitive 0.72
CYP2B6	7-benzyloxyresorufin *O*-debenzylation	orphenadrine	-	No inhibition	Competitive 200
CYP2C9	tolbutamide methyl-hydroxylation	sulfaphenazole	-	No inhibition	Mixed: Competitive 24/Uncompetitive 61
CYP2C19	*(S)* mephenytoin 4'-hydroxylation	ticlopidine	-	No inhibition	Complete inhibition
CYP2D6	dextromethorphan *O*-demethylation	quinidine	-	No inhibition	Competitive 0.046
CYP2E1	chlorzoxazone 6-hydroxylation	4-methylpyrazole	1788	Competitive inhibition	Mixed: Competitive 16/Uncompetitive 7.4
CYP3A	testosterone 6β-hydroxylation	ketoconazole	-	No inhibition	Competitive 0.044
UGT	7-hydroxycoumarin glucuronidation	-	-	No inhibition	-

Conclusion:
- FTC was not an inhibitor for human CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A
- FTC did not show inhibition of 7-hydroxycoumarin glucuronidation

11.2.2. AD-162-2005: In Vitro Assessment of Induction Potential in Metabolizing Enzymes

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-9019 in Humans	Metabolism	FTC	AD-162-2005

Methods: The potential for induction of human drug metabolizing enzymes and transporters through the activation of the aryl hydrocarbon receptor (AhR) and the pregnane X receptor (PXR) by GS-9019 was assessed in vitro. Assessments of induction were done using Puracyp's hepatoma-derived cell lines, DRE12.6 and DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. DRE12.6 cells are transformed with an expression vector for human AhR and the Drug/Dioxin Response Element (DRE) of the human CYP1A2 gene linked to a luciferase reporter. Following 24 h of exposure to the test articles, medium was replaced with phosphate-buffered saline and MultiTox-Fluor™ Multiplex assay buffer at 1:1 ratio. The plates were incubated for a further 1 hr and fluorescence determined in a Perkin-Elmer Victor 2 fluorometer (excitation 400 nm, emission 510 nm). Triplicate values were averaged and the fold induction determined by comparison with the appropriate DMSO vehicle control concentration.

	Human PXR Activation		Human AhR Activation	
	Fold Induction Over 0.1% DMSO Control		Fold Induction Over 0.1% DMSO Control	
Concentration (µM)	FTC	Rifampicin	FTC	β-Naphthoflavone
0.1	–	3.02	–	1.73
0.15	0.84	–	1.00	–
0.5	0.88	4.57	0.96	3.69
1.0	–	6.30	–	5.54
1.5	0.84	–	0.91	–
5	0.83	13.21	0.88	25.04
10	–	13.80	–	35.69
15	1.22	–	0.91	–
20	–	14.58	–	50.07
50	1.44	–	1.14	–

11.3. TAF and TFV

11.3.1. AD-120-2003: Human Cytochrome P450 Inhibition Potential of TAF

Report Title:	Study Type	Test Article	Report Number
In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-7340	Metabolism	TAF	AD-120-2003

Methods: The inhibitory effect of TAF on human P450 enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) was investigated using human liver microsomes in the presence of NADPH at concentrations of TAF up to 25 µM

Enzyme	Activity	Calculated IC_{50} (µg/mL)	
		TAF	Control Inhibitor[a]
CYP1A2	Phenacetin O-deethylase	>25	0.05
CYP2B6	Bupropion 4-hydroxylase	>25	0.72
CYP2C8	Paclitaxel 6α-hydroxylase	>25	0.34
CYP2C9	Tolbutamide 4-hydroxylase	>25	0.64
CYP2C19	(S) Mephenytoin 4'-hydroxylase	>25	7.65
CYP2D6	Dextromethorphan O-demethylase	>25	0.05
CYP3A	Midazolam 1'-hydroxylase	7.6	0.03
	Testosterone 6β-hydroxylase	7.4	0.11

CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide

a Control inhibitors: CYP1A2, α Naphthoflavone (0-3 µM); CYP2B6, Ticlopidine (0-10 µM); CYP2C8, Montelukast (0-3 µM); CYP2C9, Sulfaphenazole (0-10 µM); CYP2C19, Tranylcypromine (0-50 µM); CYP2D6, Quinidine (0-3 µM); CYP3A, Ketoconazole (0-3 µM)

11.3.2. V990172-104: Human Cytochrome P450 Inhibition Potential of TFV

Report Title:	Study Type	Test Article	Report Number
The Effect of TFV and TDF on the Activities of the Cytochrome P-450 Isoforms in Human Hepatic microsomes	Metabolism	TFV	V990172-104

Method: Probe activities selective for the CYP enzymes were utilized to examine the potential inhibitory effects of TFV using human hepatic microsomal fraction as the catalyst. Activities were evaluated in the presence and absence of 100 µM TFV.

CYP Enzyme (Activity)	Control (nmol/mg/min)	TFV (nmol/mg/min)
CYP3A (terfenadine hydroxylation)	0.018 ± 0.009	0.016 ± 0.009
CYP2D6 (dextromethorphan O-demethylation)	0.066 ± 0.041	0.064 ± 0.043
CYP2C9 (tolbutamide 4-hydroxylation)	0.218 ± 0.093	0.216 ± 0.096
CYP2E1 (chlorzoxazone 6-hydroxylation)	1.48 ± 0.58	1.50 ± 0.65
CYP1A2 (7-ethoxycoumarin O-deethylation)	0.481 ± 0.182	0.487 ± 0.19

CYP = cytochrome P450 enzyme; TFV = tenofovir

11.3.3. AD-120-2040: Human CYP Mechanism-Based Inhibition of TAF

Report Title:	Study Type	Test Article	Report Number
In Vitro Assessment of Human Hepatic Microsomal Cytochrome P450 Mechanism Based Inhibition Potential of GS-7340	Metabolism	TAF	AD-120-2040

Methods: The potential for test compound to act as a mechanism based inhibitor of human hepatic microsomal cytochromes P450 drug metabolizing enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6, was assessed. Two-stage incubation protocol was used, with the first stage allowing inactivation of the enzyme in the absence of substrate, and the second stage being used to assay remaining enzyme activity. A 10-fold dilution was performed between the 2 stages to reduce the direct inhibitory effects of the test compounds. The enzyme-specific metabolites were quantified by LC-MS/MS, except for resorufin (CYP1A2 metabolite), which was quantified fluorometrically.

Test Compound	Probe Activity	Calculated % Change	
		Control Inhibitor[a]	TAF
CYP1A2	Ethoxyresorufin O-deethylase	71.2 ± 1.9 57.9 ± 3.1	-5.61 ± 9.16
CYP2B6	Bupropion 4-hydroxylase	85.4 ± 0.8	-5.29 ± 3.52
CYP2C8	Paclitaxel 6α-hydroxylase	60.3 ± 4.8	17.4 ± 14.2
CYP2C9	Diclofenac 4'-hydroxylase	79.6 ± 2.5	-7.36 ± 17.2
CYP2C19	S-Mephenytoin 4'-hydroxylase	62.3 ± 0.9	5.30 ± 4.10
CYP2D6	Dextromethorphan O-demethylase	80.1 ± 3.6	3.94 ± 16.1
CYP3A	Midazolam 1'-hydroxylase	60.1 ± 1.8 80.2 ± 1.2	16.4 ± 5.87
	Testosterone 6β-hydroxylase	91.3 ± 0.5 70.7 ± 1.2	10.3 ± 4.66

CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide

a CYP1A2, furafylline and resveratrol; CYP2B6, ticlopidine; CYP2C8, gemfibrozil glucuronide; CYP2C9, tienilic acid; CYP2C19, ticlopidine; CYP2D6, paroxetine; CYP3A, mibefradril and mifepristone

11.3.4. AD-120-2005: Induction of Metabolizing Enzymes by TAF In Vitro

Report Title:	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-7340 in Humans	Metabolism	TAF	AD-120-2005

Methods: Assessments of induction were done using Puracyp's hepatoma-derived cell line DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. Following 24 hours of exposure to the test articles, the luciferase substrate was added and the luminescence was read in a luminometer. The average luminescent units for the 3 replicates were divided by the average for the DMSO solvent control to determine the fold induction

Concentration	Fold Induction Over 0.1% DMSO Control (PXR Activation)	
	TAF	Rifampicin
0.1 μM	NA	1.56
0.15 μM	0.81	NA
0.5 μM	0.92	4.59
1.0 μM	NA	6.36
1.5 μM	0.88	NA
5 μM	1.03	12.5
10 μM	NA	13.4
15 μM	1.58	NA
20 μM	NA	12.6
50 μM	3.89	NA

TAF = tenofovir alafenamide; NA = not applicable

Report Title:	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-7340 in Humans	Metabolism	TAF	AD-120-2005

Concentration	Fold Induction Over 0.1% DMSO Control (AhR Activation)	
	TAF	β-Naphthoflavone
0.1 μM	NA	2.56
0.15 μM	1.09	NA
0.5 μM	1.04	6.37
1.0 μM	NA	11.1
1.5 μM	0.97	NA
5 μM	0.91	47.5
10 μM	NA	40.0
15 μM	0.87	NA
20 μM	NA	27.7
50 μM	0.90	NA

TAF = tenofovir alafenamide; NA = not applicable

11.3.5. AD-120-2032: Assessment of Induction Potential of TAF in Human Hepatocyte In Vitro

Report Title:	Study Type	Test Article	Report Number
Evaluation of Induction Potential of GS-7340 in Cultured Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2032

Methods: The induction potential of cytochrome P450 (CYP) isoforms, P-glycoprotein (P-gp), and UDP glucuronosyl transferase (UGT) 1A1 by TAF was assessed in cultured human hepatocytes. GS-7340 was incubated in 3 preparations of cryopreserved human hepatocyte cultures at concentrations of 1, 10, and 100 µM with vehicle control and appropriate positive controls. Following 3 days of exposure, induction was determined *in situ* by catalytic activity assays, and mRNA expression was determined using real-time PCR (RT-PCR). Additionally, the cytotoxic potential of GS-7340 was assessed using the MTT assay.

Test Compound Concentration (µM)	Fold Induction							
	mRNA Expression in Human Hepatocytes					Human Hepatocytes		
	CYP1A2	CYP2B6	CYP3A4	P-gp	UGT1A1	CYP1A2	CYP2B6	CYP3A
1	1.2 ± 0.14	0.95 ± 0.17	0.92 ± 0.098	1.0 ± 0.037	0.94 ± 0.074	1.0 ± 0.20	1.1 ± 0.047	0.97 ± 0.083
10	3.0 ± 0.47	1.6 ± 0.26	8.3 ± 1.4	1.1 ± 0.11	1.7 ± 0.15	1.4 ± 0.15	0.85 ± 0.085	0.99 ± 0.057
100	6.9 ± 0.86	2.5 ± 0.21	44 ± 3.6	0.87 ± 0.093	3.9 ± 0.47	0.84 ± 0.065	0.42 ± 0.095	0.37 ± 0.025

TAF = tenofovir alafenamide
Note: Positive control for CYP1A2 was omeprazole, for CYP2B6 and P-gp was phenobarbital, for CYP3A was rifampicin, and for UGT1A1 was β-naphthoflavone.

Report Title:	Study Type	Test Article	Report Number
Evaluation of Induction Potential of GS-7340 in Cultured Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2032

Methods: The induction potential of cytochrome P450 (CYP) isoforms, P-glycoprotein (P-gp), and UDP glucuronosyl transferase (UGT) 1A1 by TAF was assessed in cultured human hepatocytes. GS-7340 was incubated in 3 preparations of cryopreserved human hepatocyte cultures at concentrations of 1, 10, and 100 µM with vehicle control and appropriate positive controls. Following 3 days of exposure, induction was determined in *situ* by catalytic activity assays, and mRNA expression was determined using real-time PCR (RT-PCR). Additionally, the cytotoxic potential of GS-7340 was assessed using the MTT assay.

Test Compound Concentration (µM)	Cell Viability (% Control Incubations)[a]			
	Hepatocyte Lot 228	Hepatocyte Lot 307	Hepatocyte Lot 321	Mean
0	100 ± 6	100 ± 14	100 ± 7	100 ± 9
1	104 ± 3	120 ± 10	104 ± 6	109 ± 6
10	58 ± 2	90 ± 13	81 ± 2	76 ± 6
100	47 ± 1	73 ± 8	54 ± 2	58 ± 4

TAF = tenofovir alafenamide
a Values are the mean ± standard deviation of triplicate determinations and represent percentage viability relative to solvent vehicle-only treated cells

11.3.6. AD-120-2006: In Vitro Assessment of Human UGT1A1 Inhibition Potential of TAF

Report Title:	Study Type	Test Article	Report Number
In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-7340	Metabolism	TAF	AD-120-2006

Methods: The potential for TAF to inhibit the catalytic activity of human UGT1A1 was assessed. The rates of formation of β-estradiol-3-glucuronide from estradiol substrate by hepatic microsomal fractions were determined in the presence and absence of TAF, and, where possible, IC_{50} values were determined. Silybin was used as positive control.

Enzyme	Activity	Calculated IC_{50} (μM)	
		TAF	Silybin
UGT1A1	β-estradiol-3-glucuronidation	>50	1.69

TAF = tenofovir alafenamide; UGT = uridine diphosphate glucuronosyl transferase

12. PHARMACOKINETICS: EXCRETION

12.1. BIC

12.1.1. AD-141-2303: Excretion in Mice Following Oral Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Transgenic Mice	Excretion	[^{14}C]BIC	AD-141-2303

Species	Transgenic (Ras H2 mice [CBYB6F1-Tg (HRAS) 2Jic] Mice
Gender /No. of Animals	Male/4
Feeding Condition	Non-Fasted
Vehicle/Formulation	5% Ethanol, 55% polyethylene glycol 300 and 40% water
Method of Administration	Oral Gavage
Dose	2 mg/kg (300 µCi/kg)
Specific Activity	55.9 mCi/mmol
Specific Activity of Formulation	124 µCi/mg
Analyte	Carbon-14
Assay	Liquid scintillation counting

	Mean Cumulative Recovery of % Administered ^{14}C Dose[a]		
Collection Period (h)	**Urine**	**Feces**	**Total[b]**
0–24	3.21	89.9	93.1
0–48	3.48	96.6	100
0–168	3.55	98.5	102

BIC = bictegravir (GS-9883); Non-fasted = certified rodent diet #2016C or 2016CM (Harlan) was provided ad libitum.
a Values are mean of 2 pooled samples (each from 2 animals)
b Recovery of radioactivity from cage wash, cage rinse, cage wipe and carcass (residual) totaled ~0.28% of total dose.

12.1.2. AD-141-2276: Excretion in Bile Duct-Intact Rats Following Oral Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Excretion	[^{14}C]BIC	AD-141-2276

Species	Wistar Han Rat (bile duct-intact)
Gender /No. of Animals	Male/3
Feeding Condition	Fasted
Vehicle/Formulation	5% Ethanol, 55% polyethylene glycol 300 and 40% water
Method of Administration	Oral gavage
Dose	2 mg/kg (100 µCi/kg)
Analyte	Carbon-14
Specific Activity	55.9 mCi/mmol
Specific Activity of Formulation	52.5 µCi/mg
Assay	Liquid scintillation counting

	Mean ± SD Cumulative Recovery of % Administered ^{14}C Dose		
Collection Period (h)	**Urine**	**Feces**	**Total[a]**
0–8	0.769 ± 0.206	-	0.769 ± 0.206
0–24	1.95 ± 0.39	22.9 ± 1.1	24.9 ± 1.2
0–48	3.16 ± 0.70	41.8 ± 4.5	45.0 ± 4.6
0–72	3.82 ± 0.79	56.3 ± 4.8	60.1 ± 4.9
0–96	4.25 ± 0.84	64.3 ± 5.1	68.6 ± 5.2
0–120	4.58 ± 0.84	69.8 ± 4.6	74.4 ± 4.7
0–144	4.83 ± 0.86	73.4 ± 4.0	78.2 ± 4.1
0–168	5.01 ± 0.84	76.3 ± 3.5	81.3 ± 3.6
Total Recovery (%)	95.9 ± 0.4		

BIC = bictegravir (GS-9883)
a Recovery of radioactivity from cage wash, cage rinse, and cage wipe totaled ~0.89% of total dose. Recovery of radioactivity from carcass (residual) was 13.7% of total dose.
Fasted = animals were fasted overnight prior to dose and up to 4 hours after dosing; SD = standard deviation.

12.1.3. AD-141-2298: Excretion in Bile Duct-Intact Monkeys Following Oral Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-9883 Following Oral Administration to Intact and Bile Duct-Cannulated Monkeys	Excretion	[^{14}C]BIC	AD-141-2298

Species	Intact Monkeys
Gender No. of Animals	Male/3
Feeding Condition	Fasted
Vehicle/Formulation	30% Captisol in reverse osmosis water
Method of Administration	Oral Gavage
Dose	1 mg/kg (25 µCi/kg)
Analyte	Carbon-14
Specific Activity	55.9 mCi/mmol
Specific Activity of Formulation	26.4 µCi/mg
Assay	Liquid scintillation counting

	Mean ± SD Cumulative Recovery of % Administered ^{14}C Dose		
Collection Period (h)	**Urine**	**Feces**	**Total[a]**
0–4	2.49 ± 2.00	-	2.49 ± 2.00
0-8	4.03 ± 2.26	-	4.03 ± 2.26
0–24	17.8 ± 2.6	10.7 ± 5.2	28.5 ± 5.8
0–48	19.7 ± 3.1	21.7 ± 11.6	41.4 ± 12.0
0–72	20.4 ± 3.2	33.3 ± 5.3	53.7 ± 6.2
0–96	20.6 ± 3.3	39.9 ± 3.3	60.5 ± 4.7
0–120	20.7 ± 3.3	40.5 ± 3.7	61.2 ± 5.0
0–144	20.8 ± 3.3	40.7 ± 3.7	61.5 ± 5.0
0–168	20.8 ± 3.3	40.9 ± 3.7	61.7± 5.0
Total Recovery (%)	80.4 ± 7.8		

BIC = bictegravir (GS-9883)
a Recovery of radioactivity from cage debris, cage wash, cage rinse, and cage wipe totaled ~18.7% of total dose.
Fasted = animals were fasted overnight prior to dose and up to 4 hours after dosing; SD = standard deviation.

12.2. FTC

Results from FTC excretion studies are described in Sections 5.2.1, 8.2.1, 8.2.2, and 8.2.3 of this document.

12.3. TAF and TFV

Results from the TAF excretion studies are described in Sections 5.3.1, 5.3.2, and 13.2.1 of this document.

12.3.1. 96-DDM-1278-001: Effect of Dose on the Recovery of Radioactivity Following Administration of [^{14}C]TFV to Rats

Report Title	Study Type	Test Article	Report Number
Effect of Dose on Recovery of [^{14}C]PMPA Following Intravenous Administration to Sprague-Dawley Rats	Excretion	TFV or TDF	96-DDM-1278-001

Species	Rat
Sex (M/F) No. of Animals	6M (4M group 1, 2M group 2)
Feeding Condition	Fasted
Vehicle/Formulation	Sterile saline or phosphate buffered saline
Method of Administration	IV single bolus
Dose (mg/kg/day)	10 (group 1), 50 (group 2)
Analyte (Radionuclide)	Total radioactivity, % recovery ^{14}C
Specific Activity	400 μCi/kg
Assay	Liquid scintillation counting

Excretion Route	**Urine (+ cage wash) (% dose in sample)**	**Feces (% dose in sample)**	**% Total recovery**	
Time			**Urine**	**Feces**
10 mg/kg				
0–24 h	85.2	3.18		
24–168 h	7.6	1.30	92.7	4.48
50 mg/kg				
0–24 h	77.5	7.39		
24–168 h	6.5	1.07	84.0	8.46

13. PHARMACOKINETICS: EXCRETION INTO BILE

13.1. BIC

13.1.1. AD-141-2283: Excretion in Bile Duct-Cannulated Rats Following IV Administration of BIC

Report Title Pharmacokinetics of GS-9883 Following IV Infusion to Male Bile Duct Cannulated Rats	**Study Type** Excretion	**Test Article** BIC	**Report Number** AD-141-2283
Species	Sprague-Dawley Rat (Bile Duct-Cannulated)		
Gender / No. of Animals	Male/3		
Feeding Condition	Fasted		
Vehicle / Formulation	5% ethanol, 55% polyethylene glycol 300 and 40% water		
Method of Administration	IV Infusion		
Dose (mg/kg)	0.5		
Analyte	BIC		
Assay	LC-MS/MS		
	Mean ± SD % Administered Dose		
Collection Period (h)	**Bile**	**Urine**	**Total**
0–12	0.03 ± 0.02	0.50 ± 0.88	–
0–24	0.03 ± 0.02	0.60 ± 0.79	–
0-48	0.03 ± 0.02	0.64 ± 0.85	–
0–72	0.03 ± 0.02	0.64 ± 0.85	0.67 ± 0.85

BIC = bictegravir (GS-9883); SD = standard deviation
Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing.

13.1.2. AD-141-2276: Excretion in Bile Duct-Cannulated Rats Following Oral Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats	Excretion	[^{14}C]BIC	AD-141-2276

Species	Wistar Han Rat (bile duct-cannulated)
Gender /No. of Animals	Male/3
Feeding Condition	Fasted
Vehicle/Formulation	5% Ethanol, 55% polyethylene glycol 300 and 40% water
Method of Administration	Oral Gavage
Dose	2 mg/kg (100 µCi/kg)
Analyte	Carbon-14
Specific Activity	55.9 mCi/mmol
Specific Activity of Formulation	52.5 µCi/mg
Assay	Liquid scintillation counting

	Mean ± SD Cumulative Recovery of % Administered ^{14}C Dose			
Collection Period (h)	**Bile**	**Urine**	**Feces**	**Total[a]**
0–2	3.87 ± 1.79	-	-	3.87 ± 1.79
0-4	5.10 ± 2.43	-	-	5.10 ± 2.43
0-6	6.08 ± 2.91	-	-	6.08 ± 2.91
0-8	6.86 ± 3.24	-	-	6.86 ± 3.24
0-12	8.56 ± 3.84	1.88 ± 0.45	-	10.4 ± 3.9
0–24	13.0 ± 5.4	3.15 ± 0.60	9.97 ± 1.64	26.1 ± 5.7
0–48	19.6 ± 6.1	4.65 ± 0.91	21.0 ± 2.2	45.3 ± 6.6
0–72	24.0 ± 6.4	5.70 ± 1.05	28.5 ± 3.7	58.2 ± 7.5
0–96	28.0 ± 5.9	6.41 ± 1.11	34.2 ± 4.9	68.6 ± 7.8

Report Title Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-9883 Following a Single Oral Administration to Rats			**Study Type** Excretion	**Test Article** [^{14}C]BIC	**Report Number** AD-141-2276
0–120	30.6 ± 5.8	6.88 ± 1.14	37.8 ± 5.0	75.3 ± 7.7	
0–144	32.6 ± 5.5	7.22 ± 1.15	40.5 ± 4.8	80.3 ± 7.4	
0–168	34.1 ± 5.1	7.48 ± 1.19	42.4 ± 4.7	84.0 ± 7.0	
Total Recovery (%)	99.1 ± 1.0				

BIC = bictegravir (GS-9883); SD = standard deviation
a Recovery of radioactivity from cage wash, cage rinse, and cage wipe totaled ~1.37% of total dose. Recovery of radioactivity from carcass (residual) was 13.7% of total dose.
Fasted = animals were fasted overnight prior to dose and up to 4 hours after dosing.

13.1.3. AD-141-2298: Excretion in Bile Duct-Cannulated Monkeys Following Oral Administration of [^{14}C]BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-9883 Following Oral Administration to Intact and Bile Duct-Cannulated Monkeys	Excretion	[^{14}C]BIC	AD-141-2298

Species	Bile Duct-Cannulated Monkeys
Gender /No. of Animals	Male/3
Feeding Condition	Fasted
Vehicle/Formulation	30% Captisol in reverse osmosis water
Method of Administration	Oral Gavage
Dose	1 mg/kg (25 µCi/kg)
Specific Activity	55.9 mCi/mmol
Specific Activity of Formulation	26.4 µCi/mg
Analyte	Carbon-14
Assay	Liquid scintillation counting

	Mean ± SD Cumulative Recovery of % Administered ^{14}C Dose			
Collection Period (h)	**Bile**	**Urine**	**Feces**	**Total[a]**
0-4	13.6 ± 1.6	4.13 ± 2.75	-	17.7 ± 3.2
0-8	27.5 ± 4.3	8.80 ± 5.05	-	36.3 ± 6.6
0–24	38.2 ± 7.3	13.5 ± 5.1	5.09 ± 2.14	56.8 ± 9.2
0–48	39.4 ± 7.6	14.6 ± 5.0	16.7 ± 6.1	70.7 ± 11.0
0–72	39.6 ± 7.6	14.9 ± 5.0	19.4 ± 6.4	73.9 ± 11.1
0–96	39.7 ± 7.7	15.0 ± 4.9	20.0 ± 6.4	74.7 ± 11.1
0–120	39.7 ± 7.7	15.1 ± 5.0	20.1 ± 6.4	74.9 ± 11.2
0–144	39.7 ± 7.7	15.2 ± 5.0	20.2 ± 6.4	75.1 ± 11.2
0–168	39.7 ± 7.7	15.2 ± 5.0	20.3 ± 6.5	75.2 ± 11.2

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-9883 Following Oral Administration to Intact and Bile Duct-Cannulated Monkeys	Excretion	[^{14}C]BIC	AD-141-2298
Total Recovery (%)	86.0 ± 1.7		

BIC = bictegravir (GS-9883); SD = standard deviation
a Recovery of radioactivity from cage debris, cage wash, cage rinse, cage wipe, and jacket rinse totaled ~10.7% of total dose.
Fasted = animals were fasted overnight prior to dose and up to 4 hours after dosing.

13.2. TAF and TFV

13.2.1. AD-120-2007: Excretion of [^{14}C]TAF Following Single Oral Administration in Dog

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Beagle dogs
Sex (M/F)/No. of Animals	M/3
Method of Administration	Oral gavage
Dose (mg/kg)	15
Feeding Condition	Fasted
Specific Activity	57.1 mCi/mmol
Radionuclide	Carbon-14
Vehicle/Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Tabulated PK Results :

Sample Type	Plasma	Blood
T_{max} (h)	0.25 ± 0.00	0.25 ± 0.00
C_{max} (ng eq/g)	8830 ± 774	7320 ± 895
t½ (h)	30.8 ± 4.34	107 ± 30.0
AUC_{0-t} (ng eq•h/g)	18500 ± 2020	44600 ± 11900

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	57.1 mCi/mmol
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Percent of Radioactive Dose (%)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-8 h	10.2 ± 4.17	NA	NA	10.3 ± 4.17
0-24 h	18.6 ± 4.23	29.5 ± 7.03	2.46 ± 2.33	50.6 ± 6.40
0-48 h	24.5 ± 4.30	35.0 ± 2.58	3.69 ± 2.72	63.2 ± 2.78
0-72 h	28.4 ± 4.41	35.9 ± 2.55	4.63 ± 3.29	68.9 ± 2.21
0-96 h	31.1 ± 4.46	36.4 ± 2.46	5.28 ± 3.34	72.8 ± 2.09
0-120 h	33.1 ± 4.56	36.9 ± 2.34	5.82 ± 3.48	75.8 ± 1.85
0-144 h	34.6 ± 4.63	37.2 ± 2.29	6.26 ± 3.44	78.1 ± 1.97
0-168 h	35.9 ± 4.69	37.4 ± 2.21	NA	73.3 ± 5.27

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals M/3
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Fasted
Radionuclide: Carbon-14
Specific Activity: 57.1 mCi/mmol
Vehicle / Formulation: water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)				
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Bile**	**Total**
0-1 h	NA	NA	NA	2.94 ± 2.56	2.94 ± 2.56
0-2 h	NA	NA	NA	6.80 ± 4.14	6.80 ± 4.13
0-4 h	1.72 ± 2.98	NA	NA	9.48 ± 2.24	11.2 ± 5.13
0-6 h	NA	NA	NA	12.2 ± 2.45	12.2 ± 2.43
0-8 h	8.25 ± 0.90	NA	NA	12.4 ± 2.53	20.6 ± 3.30
0-12 h	NA	NA	NA	12.5 ± 2.58	12.5 ± 2.58
0-24 h	13.9 ± 1.24	22.8 ± 19.6	0.77 ± 0.12	12.9 ± 2.70	50.3 ± 22.5
0-48 h	18.3 ± 1.50	40.8 ± 2.91	1.45 ± 0.38	13.3 ± 2.90	73.9 ± 0.90
0-72 h	21.0 ± 1.90	42.2 ± 3.61	1.81 ± 0.60	13.7 ± 3.04	78.7 ± 0.83
0-96 h	22.9 ± 1.84	42.4 ± 3.64	2.05 ± 0.63	13.8 ± 3.11	81.2 ± 0.49
0-120 h	24.5 ± 2.14	42.5 ± 3.58	2.25 ± 0.62	14.0 ± 3.16	83.3 ± 0.81
0-144 h	25.5 ± 2.29	42.6 ± 3.63	2.42 ± 0.62	14.0 ± 3.21	84.6 ± 1.02
0-168 h	26.5 ± 2.44	42.7 ± 3.64	NA	14.0 ± 3.21	83.3 ± 1.77

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

13.2.2. 96-DDM-1278-002: A Study of Biliary Excretion of [^{14}C]TFV in the Dog

Report Title	Study Type	Test Article	Report Number
A Pilot Study of Biliary Excretion of [^{14}C]PMPA in the Beagle Dog	Excretion	TFV or TDF	96-DDM-1278-002

Species	Dog
Sex (M/F) No. of Animals	1M
Feeding Condition	Fasted
Vehicle/Formulation	aqueous solution
Method of Administration	IV Bolus, single administration
Dose (mg/kg)	10
Radionuclide	^{14}C
Specific Activity	5 μCi/mg
Assay	Liquid scintillation counting

Excretion Route	Urine (% dose)	Feces (% dose)	Bile (% dose)	Cage wash (% dose)	Total (% dose)
Time					
0–48 h	70.0	0.42	0.26	5.68	76.4

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

14. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS

14.1. BIC

14.1.1. AD-141-2278: In Vitro Assessment of BIC as a P-gp or BCRP Substrate

Report Title	Study Type	Test Article	Report Number
Bi-Directional Permeability of GS-9883 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells	Drug Transport	BIC	AD-141-2278

Study System	Monolayers of P-gp- or BCRP-overexpressing MDCKII Cells in 24-well transwell plates
Method	Bi-directional permeability of BIC (10 µM) across monolayers of wild type MDCKII cells and P-gp- or BCRP-overexpressing MDCKII cells was determined in the presence and absence of known P-gp or BCRP inhibitors. BIC concentrations in the transwells were quantified by LC-MS/MS.

P_{app} (x 10^{-6} cm/sec) of BIC	Wild Type MDCKII	Pgp-overexpressing MDCKII		BCRP-overexpressing MDCKII	
		- inhibitor	+ inhibitor[a]	- inhibitor	+ inhibitor[a]
Forward (A to B)	18.6	6.3	12.5	8.1	19.0
Reverse (B to A)	23.3	47.6	30.3	52.3	38.7
Efflux Ratio	1.3	7.5	2.4	6.5	2.0

BCRP = breast cancer resistance protein; BIC = bictegravir (GS-9883); MDCKII = Madine-Darby canine kidney cell line; P-gp = P-glycoprotein; P_{app} = apparent permeability
a Control inhibitor: P-gp, cyclosporine A (10 µM); BCRP, Ko134 (10 µM)

14.1.2. AD-141-2275: In Vitro Assessment of BIC as a Substrate for OATP1B1 and OATP1B3

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of GS-9883 as a Substrate for Human OATP1B1 and OATP1B3	Drug Transport	BIC	AD-141-2275
Study System	Wild-type and human OATP1B1 and OATP1B3-transfected CHO cells		
Method	The uptake rate of BIC (1 µM) in wild type (WT) CHO cells and OAP1B1- or OATP1B3-overexpressing CHO cells was determined in the presence and absence of a known OATP inhibitor. Uptake rates were also measured with positive (atorvastatin) and negative (antipyrine) control compounds. BIC and control compound concentrations were quantified by LC-MS/MS.		

Uptake Rate (pmole/minute/1.0x10^6 cells)	BIC 1.0 µM	Atorvastatin 0.1 µM	Antipyrine 10 µM
CHO-WT	48	1.2	31
CHO-OATP1B1	43	5.1	32
CHO-OATP1B3	41	5.3	32
OATP1B1 / WT Ratio	0.9	4.4	1.0
OATP1B3 / WT Ratio	0.9	4.5	1.0

BIC = bictegravir (GS-9883); CHO = Chinese Hamster Ovary; OATP = organic anion-transporting polypeptide

14.1.3. AD-141-2273: In Vitro Inhibition of Human P-gp and BCRP by BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
In Vitro Inhibition Assessment of GS-9883 with Human P-gp and BCRP	Drug Transport	BIC	AD-141-2273

Study System	Monolayers of P-gp- or BCRP-overexpressing MDCKII cells in 96-well black cell culture plates with clear bottoms.
Method	To assess P-gp inhibition, BIC (0.33 – 80 µM) was incubated for 1 hour with P-gp-overexpressing MDCKII cells in the presence of probe substrate (calcein AM). To assess BCRP inhibition, BIC (0.33 – 80 µM) was incubated for 18 hours with BCRP-overexpressing MDCKII cells in the presence of probe substrate (pheophorbide A). Each well was analyzed for fluorescence after washing and lysing the cells. Positive control experiments were also performed in parallel with known inhibitors.

Efflux Transporter	**Substrate (Concentration)**	**IC_{50}**	
		BIC	**Control Inhibitor[a]**
P-gp	Calcein AM (10 µM)	> 80 µM	Verapamil (IC_{50} = 1.6 ± 0.3 µM)
BCRP	Pheophorbide A (1 µM)	> 80 µM	Fumitremorgin C (IC_{50} = 0.76 ± 0.04 µM)

BCRP = breast cancer resistance protein; BIC = bictegravir (GS-9883); MDCKII = Madine-Darby canine kidney cell line; P-gp = P-glycoprotein
a Control inhibitor: P-gp, verapamil; BCRP, fumitremorgin C

14.1.4. AD-141-2274: In Vitro Inhibition of Human OATP Transporters by BIC

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of GS-9883 Inhibition of Human OATP1B1 and OATP1B3	Drug Transport	BIC	AD-141-2274
Study System	CHO cells transfected with the genes encoding human OATP1B1 and OATP1B3.		
Method	BIC (0.109 – 80 µM) was incubated with OATP1B1 and OATP1B3 overexpressing cells for 1 h in the presence of probe substrate (Fluo 3). Each well was analyzed for Fluo 3 fluorescence after washing and lysing the cells. Positive control experiments were also performed in parallel with a known inhibitor.		

	Uptake Transporters IC_{50} (µM)	
Transporters	**OATP1B1**	**OATP1B3**
BIC	> 80 µM	> 80 µM
Rifampicin	1.6 ± 0.6	0.49 ± 0.19

BIC = bictegravir (GS-9883); CHO = Chinese Hamster Ovary; OATP = organic anion-transporting polypeptide

14.1.5. AD-141-2285: In Vitro Inhibition of Human OCT2 and MATE1 Transporters by BIC

Report Title	**Study Type**	**Test Article**	**Report Number**
In Vitro Assessment of GS-9883 Inhibition of Human OCT2 and MATE1	Drug Transport	BIC	AD-141-2285
Study System:	OCT2-overexpressing MDCKII cells or MATE1-overexpressing CHO cells		
Method:	To assess OCT2 inhibition, BIC (0.014 - 10 µM) was incubated for 10 minutes with non-transfected or OCT2-transfected cells in the presence of probe substrate ([^{14}C]TEA, 20 µM). To assess MATE1 inhibition, BIC (0.1 - 80 µM) was incubated at 37ºC for 10 minutes with non-transfected or MATE1-transfected CHO cells in the presence of [^{14}C]TEA. The amount of [^{14}C]TEA inside the cells was determined by liquid scintillation counter.		

Efflux Transporter	**Maximum inhibition (BIC concentration)**	**IC_{50}**
OCT2	94% (10 µM)	0.42 µM
MATE1	79% (80 µM)	8.0 µM

BIC = bictegravir (GS-9883); CHO = Chinese Hamster Ovary; MATE = multidrug and toxin extrusion transporter; MDCKII = Madine-Darby canine kidney cell line; OCT = organic cation transporter; TEA = tetraethylammonium-chloride

14.1.6. AD-141-2310: In Vitro Inhibition of Human OAT1, OAT3, OCT1 and BSEP Transporters by BIC

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition Study of GS-9883 with the Human OAT1, OAT3, OCT1 and BSEP Transporters	Drug Transport	BIC	AD-141-2310

Method:	To measure OAT1 and OCT1transporter inhibition, increasing concentrations of BIC (0.14 - 100 µM) were incubated with transporter overexpressing CHO cells in the presence of probe substrates ([^{3}H]p-aminohippuric acid, 5 µM) for OAT1, and [^{14}C]tetraethylammonium chloride (5 µM) for OCT1. For OAT3 inhibition, increasing concentration of BIC (0.14 – 100 µM) was incubated with OAT3-overexpressing Flp-In293 cell in the presence of probe substrate ([^{3}H]estrone-3-sulfate, 1 µM). Transporter specific accumulation of the probe substrate in the cells was measured and compared to its accumulation in the absence of BIC under the same assay conditions. For BSEP inhibition, BIC (0.1 – 100 µM) was incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate [^{3}H]taurocholate (2 µM) in the absence or presence of ATP. Reaction mixtures were preincubated for 15 minutes at 37°C. Cyclosporine A (20 µM) was used as positive control inhibitor and tested in parallel. Control membranes lacking transporter expression were used as negative control. All assays were performed in duplicate.

	Uptake Transporter Inhibition	
Transporter	**Maximum inhibition at 100 µM BIC (% of control)**	**IC_{50} (µM)**
OAT1	No inhibition	>100
OAT3	64	55
OCT1	13	>100
BSEP	46	>100

BIC = bictegravir (GS-9883); BSEP = bile salt export pump; CHO = Chinese hamster ovary; OAT = organic anion transporter; OCT = organic cation transporter

14.1.7. AD-141-2313: Drug-Drug Interaction Liability Assessment for BIC

Report Title	Study Type	Test Article	Report Number
Drug-Drug Interaction Liability Assessment for Bictegravir	Drug-drug interaction	BIC	AD-141-2313

Method:	The liability for BIC to cause drug interactions was assessed by calculating concentrations of BIC relevant for the interactions, compiling the enzyme and transporter interaction parameters determined in vitro, defining properties of the various victim enzymes and and victim drugs, and then calculating the various Guidance metrics and comparing the results with the threshold values in the Guidance documents.

	BIC Primary Values		
Parameter	**Identity**	**Value**	**Source**
MW	Molecular weight	449.4 g/mol	-
Dose	Maximum dose strength	50 mg (111.26 μmol)	GS-US-380-1489/1490
k_a	Absorption rate constant	2.54 hr^{-1}	BIC population PK report
F_a	Fraction of dose absorbed	1.0	Guidance default
F_g	Fraction of absorbed dose reaching portal vein	1.0	Guidance default
C_{max}	Steady state maximum plasma concentration	6.15 μg/mL (13.7 μM)	BIC population PK report
f_u	Unbound fraction in human plasma	0.25%	AD-141-2287
BPR	Whole blood to plasma concentration ratio	0.64	AD-141-2312

	Calculated BIC Concentrations Used for Drug Interactions
Concentration	**Value**
C_{max} or $[I]_1$ or $[I_1]$	13.7 μM
$C_{max,u}$	0.034 μM / 0.137 μM[a]
$[I]_{gut}$ or $[I]_g$ or $[I]_2$ or $[I_2]$	445.0 μM

Report Title	Study Type	Test Article	Report Number
Drug-Drug Interaction Liability Assessment for Bictegravir	Drug-drug interaction	BIC	AD-141-2313

$[I]_g$	15.7 µM
$[I]_h$ or $[I]_{u,inlet,max}$	0.046 µM / 0.117 µM[a]
$f_u \times [I]_{in,max}$	FDA: 0.042 µM / 0.168 µM[a]
$f_u \times [I]_{inlet,max}$	PMDA: 0.042 µM / 0.166 µM[a]

	Calculations for Reversible CYP Inhibition by BIC[b]			
Enzyme	**Intestinal**	**Hepatic**	**AUCR**	**EMA Metric[a]**
CYP1A2	1.0 ≥ Value > 0.75	1.00	1.00	< 0.001 / < 0.003
CYP2B6	1.0 ≥ Value > 0.76	1.00	1.00	< 0.001 / < 0.003
CYP2C8	1.0 ≥ Value > 0.75	1.00	1.00	< 0.001 / < 0.003
CYP2C9	1.0 ≥ Value > 0.71	1.00	1.00	< 0.001 / < 0.004
CYP2C19	1.0 ≥ Value > 0.73	1.00	1.00	< 0.001 / < 0.003
CYP2D6	1.0 ≥ Value > 0.73	1.00	1.0 ≤ Value < 1.06	< 0.001 / < 0.003
CYP3A M	1.0 ≥ Value > 0.75	1.00	1.0 ≤ Value < 1.12	< 0.001 / < 0.003
CYP3A T	1.0 ≥ Value > 0.73	1.00	1.0 ≤ Value < 1.13	< 0.001 / < 0.003

	Calculations for Induction Liability for BIC						
	Hepatocyte Data			Basic	Net Effect		
Target	**$EC_{50,u}$ (µM)**	**E_{max}[c]**	**d**	**R_3 or R**	**Intestinal**	**Hepatic**	**AUCR**
CYP1A2	No induction	16.3	0.90	≥ 0.9	1.0	1.0	1.0
CYP2B6	102.8	10.5	1.04	**0.45**	2.39	1.00	1.0
CYP3A4	19.1	22.7	0.40	**0.21** / **0.10**[d]	5.1 / 11.2[d]	1.02 / 1.05[d]	**0.36** / **0.16**[d]

Report Title	Study Type	Test Article	Report Number
Drug-Drug Interaction Liability Assessment for Bictegravir	Drug-drug interaction	BIC	AD-141-2313

	Calculations for Intestinal Efflux Transporter Interactions for BIC				
Transporter	**$IC_{50,u}$ (µM)**	**$K_{i,u}$ (µM)**	**FDA $[I]_2/K_{i,u}$**	**EMA 0.1 × $[I]_{gut}$ (µM)**	**PMDA $[I_2]/K_{i,u}$**
P-gp	> 80	> 40	**11.1**	**44.5**	**11.1**
BCRP	> 80	> 40	**11.1**	**44.5**	**11.1**

	Calculations for Hepatic Uptake Transporter Interactions for BIC					
Transporter	**$IC_{50,u}$ (µM)**	**$K_{i,u}$ (µM)**	**FDA $[I]_1/K_{i,u}$**	**FDA R**	**EMA 25 × $[I]_h$ (µM)**	**PMDA R**
OATP1B1	Not an inhibitor		< 0.1	< 1.25	2.9	< 1.25
OATP1B3	> 80	> 40	**< 0.34**	1.0	2.9	1.0
OCT1	> 100	> 50	**< 0.27**	1.0	2.9	1.0

	Calculations for Hepatic Efflux Transporter and Renal Transporter Interactions for BIC					
Transporter	**$K_{i,u}$ (µM)**	**FDA $[I]_1/K_{i,u}$**	**FDA $C_{max,u}/K_{i,u}$[a]**	**EMA 50 × $C_{max,u}$ (µM)[a]**	**PMDA $[I_1]/K_{i,u}$**	**PMDA 1 + $C_{max,u}/K_{i,u}$[a]**
OAT1	Not an inhibitor	NA	< 0.1	1.7 / 6.9	NA	< 1.25
OAT3	27.5	NA	0.001 / 0.005	1.7 / 6.9	NA	1.001 / 1.005
OCT1	> 50	NA	< 0.001 / < 0.003	1.7 / 6.9	NA	< 1.001 / < 1.003
OCT2	0.21	NA	**0.16** / **0.65**	**1.7** / **6.9**	NA	1.16 / **1.65**
MATE1	4.0	NA	0.01 / 0.03	1.7 / **6.9**	NA	1.01 / 1.03
Pgp	> 80	**< 0.34**	NA	1.7 / 6.9	**< 0.34**	NA
BCRP	> 80	**< 0.34**	NA	1.7 / 6.9	**< 0.34**	NA
BSEP	> 100	**< 0.27**	NA	1.7 / 6.9	**< 0.27**	NA

BIC = bictegravir (GS-9883); NA = not applicable (transporter does not fall in that classification)
Values exceeding the respective threshold are in **bold**

a Value calculated using plasma f_u = 0.25% / value calculated using plasma f_u = 1.0%
b Calculated R values failed using the FDA basic models
c Corrected for baseline (1-fold) increase in mRNA
d Value calculated using d = 0.40 / value calculated using d = 1.0

14.2. FTC

Results from FTC transporter related drug-drug interaction studies are described as a component of STB (EVG/COBI/FTC/TFV) in Section 14.4.

14.3. TAF and TFV

14.3.1. AD-120-2018: Bidirectional Permeability of TAF Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells

Report Title:	Study Type	Test Article	Report Number
Bidirectional Permeability of GS-7340 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TAF	AD-120-2018

Methods: The potential for TAF to act as a substrate for P-gp (MDR1) and BCRP was tested in monolayers of either wild type, MDR1-transfected or BCRP-transfected Madin-Darby canine kidney (MDCK II) cells (MDCK II-WT, MDCK II-MDR1 and MDCK II-BCRP, respectively). The effects of transporter-selective inhibitors were also assessed.

Wild Type and MDR1 Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (× 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCK II-WT	Cell-Free	7.9	112	45.7	NA	45.7	NA
	Forward	7.9	103	1.4	1.5	1.5	4.8
	Reverse	7.6	99	6.7	7.4	7.1	
MDCK II-MDR1	Forward	7.4	92	0.1	0.3	0.2	66.2
	Reverse	7.7	96	12.8	11.3	12.1	
MDCK II-MDR1 (10 µM Cyclosporin A)	Forward	9.4	91	1.3	1.2	1.3	5.6
	Reverse	8.4	90	6.8	7.4	7.1	

TAF = tenofovir alafenamide; MDR1 = P-glycoprotein (P-gp, ABCB1); NA = not applicable

Report Title:	Study Type	Test Article	Report Number
Bidirectional Permeability of GS-7340 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TAF	AD-120-2018

Wild Type and BCRP Transfected MDCK II Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (× 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCK II-WT	Cell-Free	7.9	112	45.7	NA	45.7	NA
	Forward	7.9	103	1.4	1.5	1.5	4.8
	Reverse	7.6	99	6.7	7.4	7.1	
MDCK II-BCRP	Forward	7.8	79	2.1	2.2	2.1	6.2
	Reverse	7.8	104	13.5	13.3	13.4	
MDCK II-BCRP (10 µM Ko134)	Forward	8.9	103	3.8	5.8	4.8	1.4
	Reverse	10.3	96	5.9	8.0	6.9	

TAF = tenofovir alafenamide; NA = not applicable

14.3.2. AD-120-2019: In Vitro Assessment of TAF Inhibition of Human OATP1B1, OATP1B3, P-gp, and BCRP

Report Title:	Study Type	Test Article	Report Number
In Vitro Assessment of GS-7340 Inhibition of Human OATP1B1, OATP1B3, P-gp, and BCRP	Drug-drug interaction (in vitro)	TAF	AD-120-2019

Methods: The inhibition potential of TAF of human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3. TAF and positive control compound were diluted in assay buffer containing 2 µM Fluo 3. Following removal of media containing Fluo 3, the cells were immediately analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm.
The inhibition potential of TAF of human P-gp and BCRP was assessed in Madin Darby Canine Kidney (MDCKII) cells, either wild type or transfected with the genes encoding human P-gp or BCRP. The incubation was carried out in cell culture medium (without FBS) containing 10 µM Calcein AM (P-gp) or 1 µM pheophorbide a (PhA) (BCRP). Following removal of media containing calcein AM or PhA, the cells were analyzed immediately for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm or PhA fluorescence at an excitation of 415 nm and an emission of 675 nm.

	Uptake Transporters IC_{50} (µM)		Efflux Transporters IC_{50} (µM)	
Test Compound	**OATP1B1**	**OATP1B3**	**P-gp**	**BCRP**
TAF	> 100	> 100	> 100	> 100
Rifampicin	2.4 ± 1.1	1.7 ± 0.4	NA	NA
Verapamil	NA	NA	3.7 ± 3.1	NA
Fumitremorgin C	NA	NA	NA	0.32 ± 0.03

BCRP = breast cancer resistance protein; NA = not applicable; P-gp = P-glycoprotein; TAF = tenofovir alafenamide

14.3.3. AD-120-2013: Effect of GS-9350 on the Bidirectional Permeability of TAF Through Caco-2 Cells

Report Title:	Study Type	Test Article	Report Number
Effect of GS-9350 on the Bidirectional Permeability of GS-7340 through Caco-2 Cells	Absorption (in vitro)	TAF	AD-120-2013

Bidirectional Permeability of TAF Through Caco-2 Cells

Inhibitor	Direction	Target Concentration (µM)	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)					Efflux Ratio
					Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	
–	Cell-Free	10	9.61	119	31.6	–	30.1	–	30.8	20
	Forward		9.92	64	0.59	0.69	0.82	0.88	0.74	
	Reverse		8.71	102	18.0	15.1	12.6	14.8	15.1	
GS-9350	Forward	10	11.0	101	3.98	3.56	2.47	2.55	3.14	1.6
	Reverse		11.4	115	6.71	6.18	3.33	3.33	4.89	

Caco-2 = human colonic adenocarcinoma cell line; TAF = tenofovir alafenamide; P_{app} = apparent permeability; GS-9350 = cobicistat (COBI)

14.3.4. AD-120-2022: In Vitro Assessment of TAF as a Substrate for Human OATP1B1 and OATP1B3

Report Title:	Study Type	Test Article	Report Number
In Vitro Assessment of GS-7340 as a Substrate for Human OATP1B1 and OATP1B3	Drug-drug interaction (in vitro)	TAF	AD-120-2022

Methods: The potential of TAF as a substrate in human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3 in the presence and absence of 40 µM rifampicin (OATP inhibitor). Following removal of media, the cell extracts were analyzed by LC-MS/MS.

Test Compound	Uptake Rate (pmole/min/million cells)		Uptake Ratio
	TAF	TAF + Rifampicin	
CHO-WT	9.0	6.0	1.5
CHO-OATP1B1	12.0	6.2	1.9
CHO-OATP1B3	24.1	5.8	4.2
OATP1B1/WT Ratio	1.3	NA	NA
OATP1B3/WT Ratio	2.7	NA	NA

NA = not applicable; OATP = organic anion transporting polypeptide (SLCO or SLC22A gene products); TAF = tenofovir alafenamide

14.3.5. AD-120-2042: Effect of an OATP Inhibitor on Uptake of TAF into Primary Human Hepatocytes

Report Title:	Study Type	Test Article	Report Number
Effect of an OATP Inhibitor on Uptake of TAF into Primary Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2042

Methods: The effect of OATP1B1 and OATP1B3 inhibitor, rifampicin on TAF uptake into primary human hepatocytes was assessed by measuring the intracellular levels of the pharmacologically active nucleotide analog diphosphate, tenofovir diphosphate (TFV-DP). Cells were pre-incubated with 20 μM rifampicin followed by incubation with 0.5 μM TAF. The amount of TFV-DP formation was analyzed by LC-MS/MS. Bosentan was used as a positive control.

Compound	Hepatocyte Donor	Mean Concentration (pmole/million cells)[a]		%Inhibition
		TAF	TAF + 20 μM Rifampicin	
TAF	1	33.3	31.7	4.8
	2	12.6	11.8	6.3
	3	16.6	11.0	34
	4	9.3	8.0	14
	Mean ± SD	17.9 ± 10.6	15.6 ± 10.8	13
Bosentan	1	64.8	40.8	37
	2	53.4	36.0	33
	3	45.8	30.3	34
	4	81.1	43.5	46
	Mean ± SD	61.3 ± 15.4	37.7 ± 5.8	38

OATP = organic anion transporting polypeptide (SLCO or SLC22A gene products); TAF = tenofovir alafenamide
a For TAF, intracellular levels of TFV-DP are reported and for bosentan, cell-associated levels are reported. Results are mean of duplicate experiments.

14.3.6. AD-120-2036: In Vitro Assessment of TAF as an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or as a Substrate for OCT1

Report Title:	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	TAF	AD-120-2036

Methods: The potential for TAF to inhibit the human organic anion and cation uptake transporters, and multidrug and toxin extrusion transporter MATE1 was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells (for OAT1, OCT1, OCT2, and MATE1), transfected Flp-In 293 cells (for OAT3). The amount of substrate inside the cells was determined by liquid scintillation/fluorescence reader.

Inhibitor or Enhancer	IC_{50} (µM)	Maximum Inhibition at 100 µM (% of Control)
OCT1	> 100	26
OCT2	> 100	NA
OAT1	> 100	8
OAT3	> 100	16
MATE1	> 100	34

BSEP = bile salt export protein; MATE1 = multidrug and toxin extrusion protein 1; NA = not applicable; OAT = organic anion transporter; OCT = organic cation transporter; TAF = tenofovir alafenamide

Report Title:	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	TAF	AD-120-2036

Methods: The potential for TAF to inhibit the bile salt export pump BSEP was assessed in vitro using transfected Chinese hamster ovary (CHO) cells. TAF was incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate, taurocholate (2 µM) in the absence or presence of ATP. The amount of substrate inside the filtered vesicles was determined by liquid scintillation reader.

Inhibitor or Enhancer	IC_{50} (µM)	Maximum Inhibition at 100 µM (% of Control)
BSEP	> 100	43

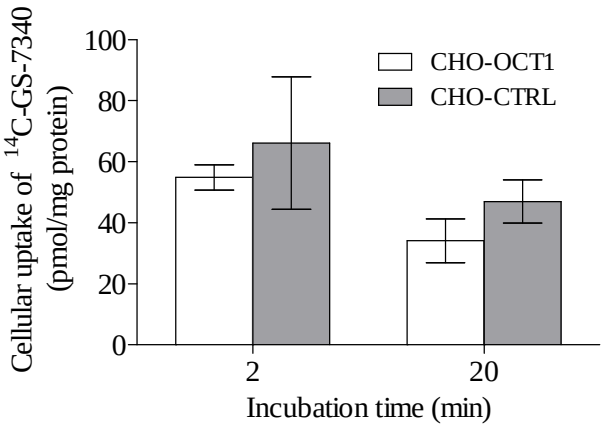
BSEP = bile salt export protein; TAF = tenofovir alafenamide

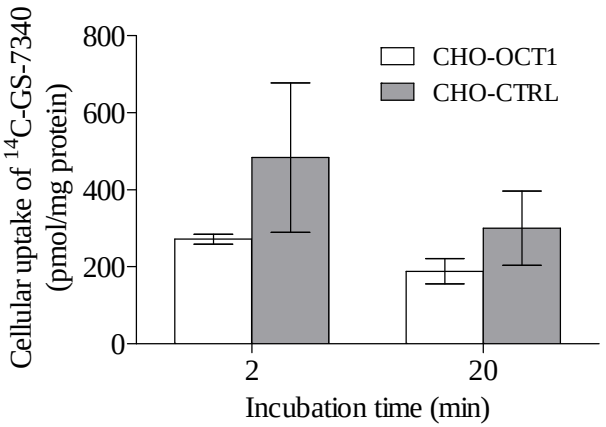
Report Title:	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	[^{14}C]TAF	AD-120-2036

Methods: The interactions of TAF with human cation transporters OCT1 was assessed in vitro using OCT1 transporter-expressed Chinese hamster ovary (CHO) cells. Transporter specific accumulation into OCT1 transporter-expressing cells was investigated at 2 concentrations (1 and 10 µM) and time points (2 and 20 minutes) to determine if TAF is a substrate for this transporter.

Results: TAF was found not to be a substrate for OCT1 based on no transporter specific accumulation of TAF in the OCT1 transporter-expressing cells.

1 µM **10 µM**

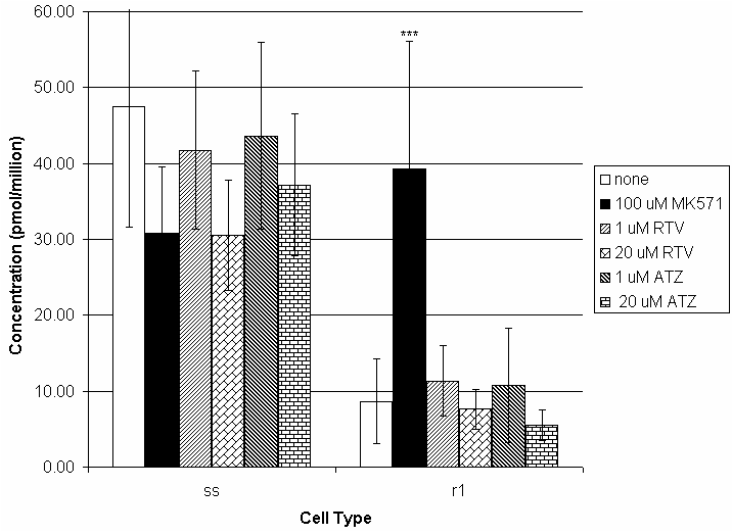




14.3.7. AD-104-2001: In Vitro Study of the Potential of TFV to be a Substrate of MRP2 and MRP4 and the Effect of the HIV Protease Inhibitors Atazanavir and Ritonavir

Report Title:	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors on the Transport of Tenofovir by the Multidrug Resistance Related Proteins 2 and 4	Drug-Drug Interaction	TFV	AD-104-2001

The effects of RTV and ATZ on the accumulation of TFV (1 µM) in MRP4 over-expression cells (r1) and parental cells (SS) were determined. It was found that > 5-fold less TFV accumulated in CEM-R1cells (MRP4 over-expressing) relative to CEM-SS parental cells following. MK571 (100 µM, a potent inhibitor of MRPs) was able to increase TFV levels in CEM-R1 cells to concentrations similar to those in CEM-SS cells. RTV and ATZ (1 and 20 µM) had no significant effect on TFV levels in CEM-SS or –R1 cells.



Statistically significant change in intracellular TFV relative to no cotreatment in CEM-R1 cells (MRP4 overexpressing). Unpaired 2-tailed student's t-test assuming equal variance (***P < 0.001).

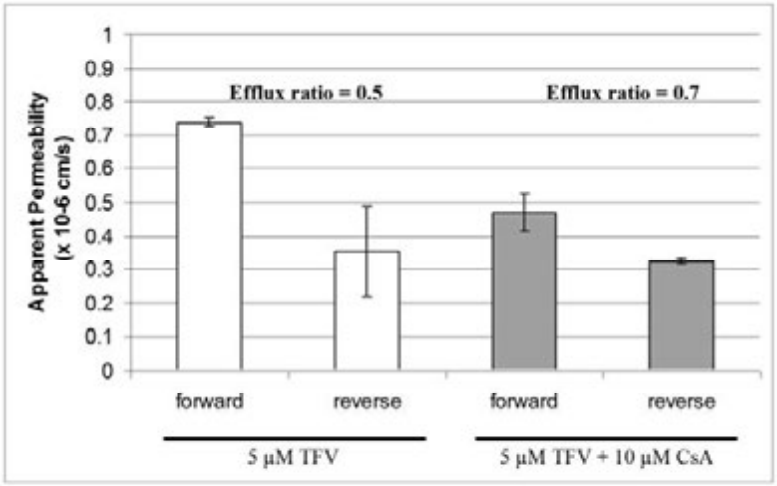
Conclusion: These findings implicate MRP4, but not MRP2, as a transporter potentially contributing to the active tubular secretion of TFV. The results of these studies also suggest that the HIV-PIs ATZ and RTV, even when tested at supra-pharmacological concentrations, are either weak or not inhibitors of transport mediated by MRP2 and MRP4.

14.3.8. AD-104-2002: In Vitro Study of the Potential of TFV to be a Substrate of P-gp and the Effect of other Drugs

Report Title:	Study Type	Test Article	Report Number
Lack of a Contribution from P-glycoprotein (P-gp) in the Active Tubular Secretion of Tenofovir	Drug-Drug Interaction	TFV or TDF or tenofovir amidate	AD-104-2002

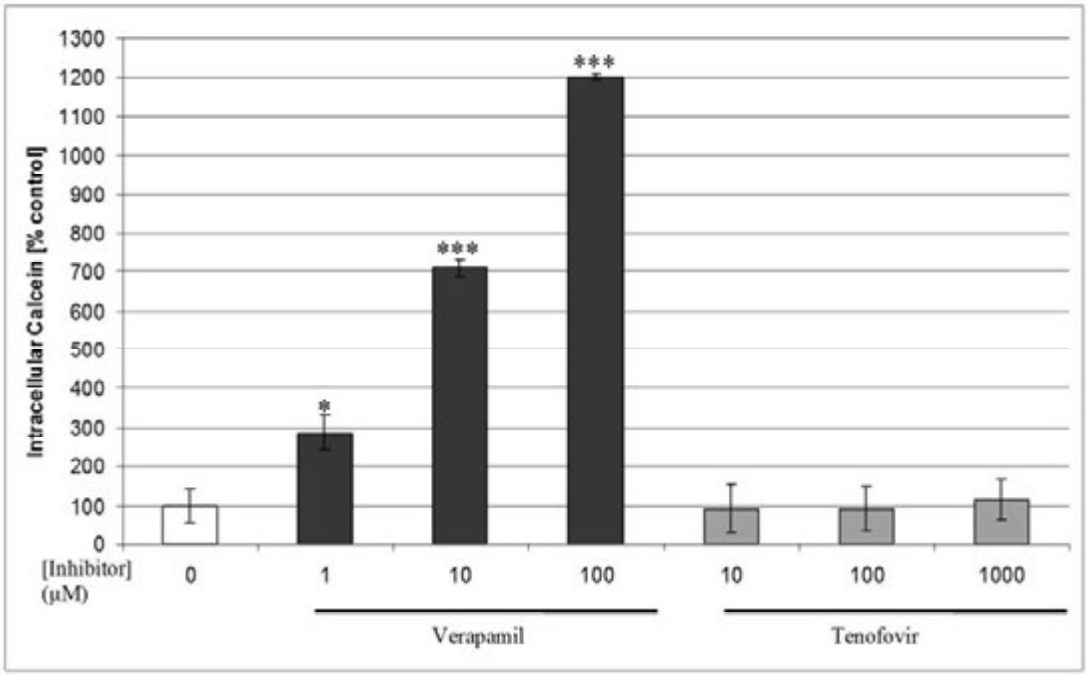
Methods: In vitro bidirectional permeability experiments with TFV (5 and 50 µM) in Caco-2 cell monolayers. In vitro inhibition studies of the accumulation of a model multidrug resistance protein (MDR1; P-glycoprotein) substrate (calcein) by TFV (up to 1000 µM) were conducted following incubation of a Madin-Darby canine kidney cell line (MDCK II), both parental and stably transfected with human P-gp, with calcein AM.

Results: The figure below shows the forward and reverse permeability of TFV through Caco-2 cell monolayers, and the calculated efflux ratio in the presence or absence of CsA. TFV had similar forward and reverse permeability that was unaffected by incubation with the P-gp inhibitor CsA (cyclosporine).



Report Title:	Study Type	Test Article	Report Number
Lack of a Contribution from P-glycoprotein (P-gp) in the Active Tubular Secretion of Tenofovir	Drug-Drug Interaction	TFV or TDF or tenofovir amidate	AD-104-2002

The figure below displays the effect of TFV and the control P-gp inhibitor, verapamil, on the accumulation of the fluorescent P-gp substrate calcein in MDCK cells transfected with human P-gp. Verapamil significantly inhibited P-gp and resulted in higher intracellular calcein levels with increasing concentration. Tenofovir at concentrations up to 1000 µM did not inhibit the P-gp efflux of calcein.



Conclusion: The observation that TFV has an efflux ratio close to 1, which is not affected by a P-gp inhibitor, in Caco-2 cell monolayers suggests that it is not a P-gp substrate. TFV did not inhibit the transport of a P-gp substrate when tested at suprapharmacological concentrations in MDCK II cells stably transfected with P-gp indicating that it is not a substrate or inhibitor of P-gp.

14.3.9. PC-104-2010: Potential for TFV to be an OAT1 Substrate and the Effect of the Other Drugs

Report Title:	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Methods: The effect of protease inhibitors and other therapeutics on the OAT1 mediated transport of [^{3}H]TFV (1.2 µM) was studied in a stable cell line (CHO-OAT1 cells). The test drugs were examined at concentrations corresponding to 3×, 1× and 0.33× their reported clinical C_{max} values.

Tested Drug	Concentration (Fold Clinical C_{max})	Transport of TFV by OAT1 [% Control]	
		Serum-free Medium	50% Human Serum[a]
None	-	100	100
Lopinavir	3×	62.5 ± 0.7	113.0 ± 19.8
	1×	87.5 ± 0.7	94.8 ± 1.8
	0.33×	96.0 ± 4.2	109.0 ± 7.1
Lopinavir/ritonavir	3×	55.0 ± 2.8	95.0 ± 5.7
	1×	86.5 ± 0.7	97.0 ± 5.7
	0.33×	109.5 ± 3.5	103.0 ± 0.0
Ritonavir	3×	73.0 ± 7.1	98.5 ± 6.4
	1×	89.5 ± 4.9	97.5 ± 7.8
	0.33×	95.5 ± 4.9	102.5 ± 13.4

Report Title:	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Tested Drug	Concentration (fold Clinical C_{max})	Transport of TFV by OAT1 [% Control]	
		Serum-free Medium	50% Human Serum[a]
Atazanavir	3×	104.0 ± 0.0	nd[b]
	1×	102.0 ± 2.8	nd
	0.33×	103.5 ± 3.5	nd
Saquinavir	3×	88.5 ± 9.2	102.5 ± 10.6
	1×	90.0 ± 8.5	96.0 ± 7.1
	0.33×	97.5 ± 2.1	98.0 ± 9.9
Nelfinavir	3×	63.5 ± 13.4	90.5 ± 6.4
	1×	78.5 ± 10.6	94.0 ± 1.4
	0.33×	95.0 ± 12.7	107.5 ± 0.7
Amprenavir	3×	78.5 ± 7.8	96.5 ± 12.0
	1×	85.0 ± 8.5	104 ± 9.9
	0.33×	98.0 ± 7.1	109 ± 8.5

a Serum protein binding: lopinavir – 98%, ritonavir – 99%, atazanavir – 86%, saquinavir – 97%, nelfinavir – 98%, amprenavir – 90%.
b nd: not determined

Report Title:	**Study Type**	**Test Article**	**Report Number**
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Tested Drug	**Concentration (fold Clinical C_{max})**	**Transport of TFV by OAT1 [% Control]**	
		Serum-free Medium	**50% Human Serum[a]**
None	-	100	100
Acyclovir	3×	91 ± 2.8	nd[b]
	1×	94 ± 2.8	nd
	0.33×	101 ± 4.2	nd
Ganciclovir	3×	101 ± 0.0	nd
	1×	92.5 ± 7.8	nd
	0.33×	102.5 ± 0.7	nd
Oseltamivir carboxylate	3×	101 ± 2.8	nd
	1×	102 ± 1.4	nd
	0.33×	118 ± 2.8	nd
Trimethoprim	3×	103.5 ± 6.4	nd
	1×	98.5 ± 4.9	nd
	0.33×	101.5 ± 0.7	nd
Sulfamethoxazole	3×	79.5 ± 6.4	91.5 ± 0.7
	1×	93.5 ± 0.7	98.5 ± 0.7
	0.33×	102 ± 1.4	112 ± 4.2

Report Title:	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors and Other Therapeutics on the Transport of Tenofovir by Human Renal Organic Anion Transporter Type 1 (OAT1)	Drug-Drug Interaction	TFV	PC-104-2010

Tested Drug	Concentration (fold Clinical C_{max})	Transport of TFV by OAT1 [% Control]	
		Serum-free Medium	50% Human Serum[a]
Amoxicillin	3×	91 ± 5.7	nd[b]
	1×	91.5 ± 3.5	nd
	0.33×	94 ± 8.5	nd
Ibuprofen	3×	7[c]	99.5 ± 2.1
	1×	19[c]	102.5 ± 0.7
	0.33×	32[c]	114.5 ±4.9
Acetaminophen	3×	78.5 ± 3.5	94.5 ± 3.5
	1×	88 ± 1.4	94.5 ± 9.2
	0.33×	97 ± 7.1	106.5 ± 3.5

a Serum protein binding: sulfomethoxazole – 70%, ibuprofen – 99%, acetaminophen – 25%.
b nd: not determined
c n = 1

14.3.10. PC-104-2011: Potential for TFV to be an OAT3 Substrate and the Effect of HIV-PIs

Report Title:	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors on the Transport of TFV by Human Renal Organic Anion Transporter Type 3 (OAT3)	Drug-Drug Interaction	TFV	PC-104-2011

Type of Study: In vitro study was conducted in appropriate cell lines expressing hOAT3 to define the role of OAT3 in tenofovir (TFV) transport and to assess the effect of HIV protease inhibitors (HIV-PIs) on the OAT3 transport of TFV.

Methods: The effect of HIV-PIs on the OAT3 mediated transport of [^{3}H]TFV (1.2 µM) was studied in a stable cell line (BHK-OAT3 cells). HIV-PIs were tested at concentrations corresponding to 2x, 1x, and 0.5x their reported clinical C_{max} values. The kinetics of [^{3}H]TFV and [^{3}H]estrone sulphate transport were also determined in BHK-OAT3 cells.

Substrate	K_m [µM]	V_{max} [pmol/10^6 cells/min]	Transport efficiency (V_{max}/K_m)
Tenofovir (n = 3)	767 ± 145	31.6 ± 8.0	0.043 ± 0.016
Estrone sulfate (n = 4)	3.0 ± 1.2	2.9 ± 1.2	0.97 ± 0.15

Report Title:	Study Type	Test Article	Report Number
Effect of HIV Protease Inhibitors on the Transport of TFV by Human Renal Organic Anion Transporter Type 3 (OAT3)	Drug-Drug Interaction	TFV	PC-104-2011

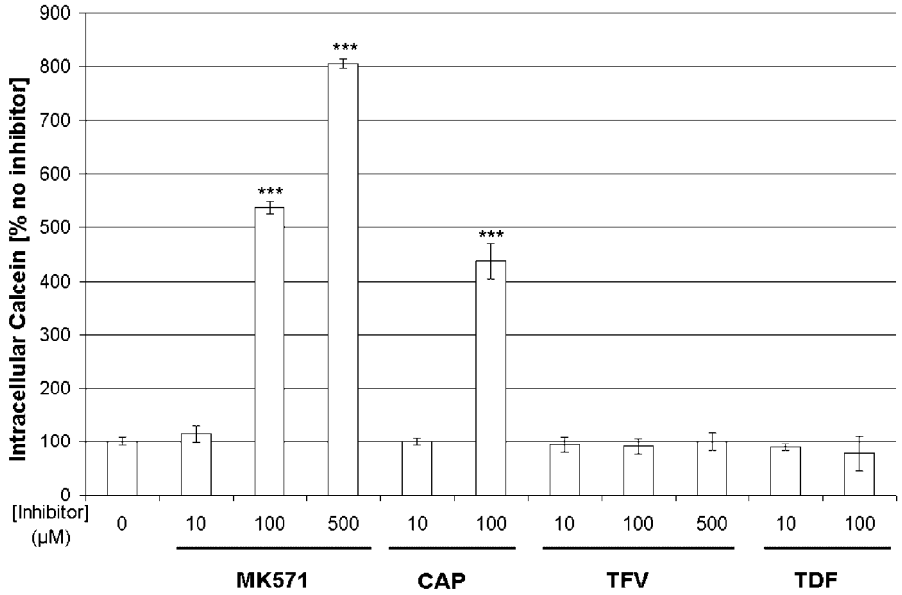
Tested PI	Concentration [fold Clinical C_{max}]	Transport of TFV by OAT3 [% Control]	
		Serum-free Medium	40% Human serum
No inhibitor	-	100	100
Ritonavir	0.5×	57.7 ± 5.1	87.1 ± 6.7
	1×	38.4 ± 5.7	65.9 ± 8.8
	2×	21.1 ± 3.2	59.4 ± 7.0
Lopinavir/ritonavir	0.5×	78.9 ± 2.6	93.3 ± 12.2
	1×	63.5 ± 8.7	92.1 ± 6.8
	2×	46.1 ± 5.1	78.5 ± 9.1
Atazanavir	0.5×	102.3 ± 1.6	103.8 ± 14.9
	1×	94.7 ± 7.8	98.5 ± 4.4
	2×	90.8 ± 2.4	97.1 ± 7.7
Nelfinavir	0.5×	84.6 ± 2.3	102.0 ± 10.2
	1×	73.1 ± 3.2	101.6 ± 11.2
	2×	47.7 ± 2.3	99.4 ± 9.8
Saquinavir	0.5×	98.3 ± 0.1	99.2 ± 8.7
	1×	95.1 ± 3.6	98.3 ± 3.7
	2×	98.8 ± 3.7	101.1 ± 13.2
Amprenavir	0.5×	87.8 ± 5.6	97.0 ± 2.7
	1×	81.4 ± 3.1	90.3 ± 5.2
	2×	78.7 ± 2.4	86.0 ± 1.5

Conclusion: TFV was found to be a low affinity substrate for OAT3 (K_M 767 uM). When adjusted for plasma protein binding and tested at their clinical C_{max} concentration, HIV-PIs had no significant effect on TFV transport by OAT3.

14.3.11. PC-104-2014: Effect of TFV on the Activity of Human Multidrug Resistance Related Protein MRP1

Report Title:	Study Type	Test Article	Report Number
Lack of a Contribution from MRPl in Tubular Re-absorption of TFV	Drug-Drug Interaction	TFV	PC-104-2014

Methods: Inhibition of the accumulation of a model MRP1 substrate (calcein) in a Madin-Darby canine kidney cell line (MDCK II) stably transfected with human MRP1, and treated with calcein-AM. Control inhibitors, MK571 and CAP (caffeic acid phenethyl ester), were tested in parallel.



Conclusion: Suprapharmacological concentrations of TFV did not inhibit the transport of an MRP1 substrate in MDCK II cells stably transfected with MRP1.

14.3.12. AD-104-2012: Effects of TFV on Transport by Human OCT2 and MATE1

Report Title:	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Tenofovir with Human OCT2 and MATE1 Transporters	Drug-Drug Interaction	TFV	AD-104-2012

Type of Study: In vitro study of the effect of TFV on the activity of the human renal transporters, OCT1 and MATE1, expressed individually in CHO cells.

Methods: The model substrate was [^{14}C]triethylamine (TEA, 3.6 µM) and cell monolayers were exposed for 5 minutes (OCT2) or 20 minutes (MATE1) before being washed and cell-associated radioactivity determined. Results were compared to those obtained in nontransfected CHO cells. Positive control inhibitors (100 µM verapamil for OCT2 and 100 µM quinidine for MATE1) were tested in parallel.

Results: In the absence of inhibitors, accumulation of TEA in transfected cells was 25-fold higher (OCT2) and 18-fold higher (MATE1) than parental CHO cells. Positive control inhibitors reduced the transporter-specific accumulation of TEA to 8.68 ± 2.56% (OCT2) and 3.70 ± 0.62% (MATE1) of the vehicle control, confirming sensitivity to inhibitors. At concentrations of TFV up to 300 µM there was little or no effect on TEA accumulation.

Inhibitor	Transporter	IC_{50} (µM)	Maximum inhibition
TFV	OCT2	> 300	< 10%
	MATE1	> 300	~20%

CHO = Chinese hamster ovary; MATE1 = multidrug and toxin extrusion protein 1; OCT2 = organic cation transporter 2

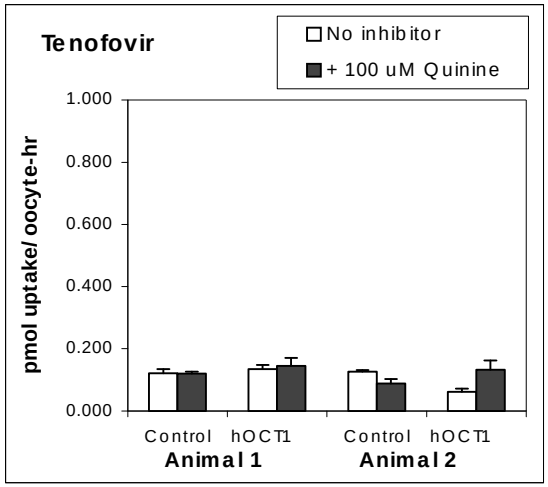
14.3.13. PC-103-2001: Interactions of TFV with Human OAT3, OCT1, and OCT2

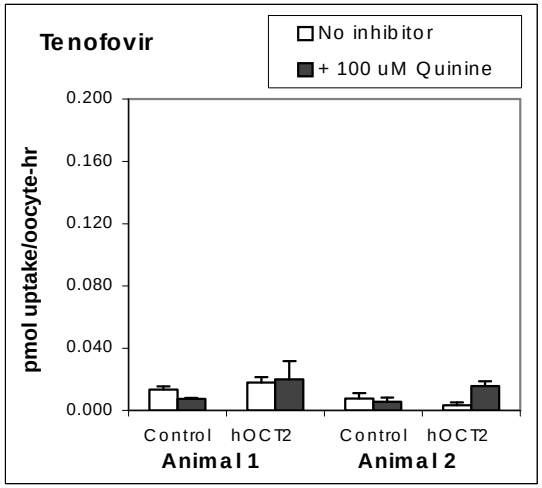
Report Title:	Study Type	Test Article	Report Number
In Vitro Interactions of Acyclic Nucleoside Phosphonate Analogs with Human Organic Cation and Anion Transporters	Drug-Drug Interaction	TFV	PC-103-2001

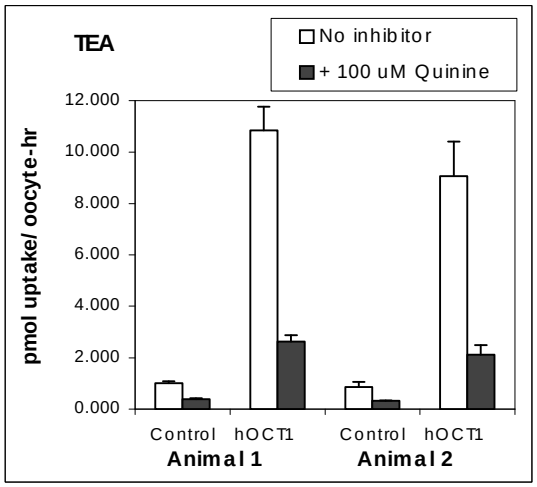
Type of Study: In vitro study to evaluate the interactions of tenofovir (TFV) with human organic anion transporter OAT3 and human cation transporters OCT1 and OCT2.

Methods: The interactions of TFV with OCT1, OCT2, and OAT3 were studied in a Xenopus oocyte expression system through uptake transport of [^{3}H]TFV (10 µM) and appropriate control substrates ([^{14}C]TEA triethylamine, 100 µM for OCT1 and OCT2, and [^{3}H]estrone sulphate (100 nM) for OAT3). Inhibition studies were also conducted for OAT3 by measuring the uptake of [^{3}H]estrone sulphate (100 µM) in the presence of TFV and the positive control inhibitor, probenicid, at concentrations from 50 to 1000 µM.

Results (OCT1 and OCT2): The figures below show the results of uptake studies of [^{3}H]TFV (10 µM) for OCT1 and OCT2 in comparison to water-injected oocytes (Control) in the presence and absence of the inhibitor quinine. The positive control substrate, TEA, showed the expected high level of transport. TFV was shown not to be a substrate for OCT1 and OCT2.

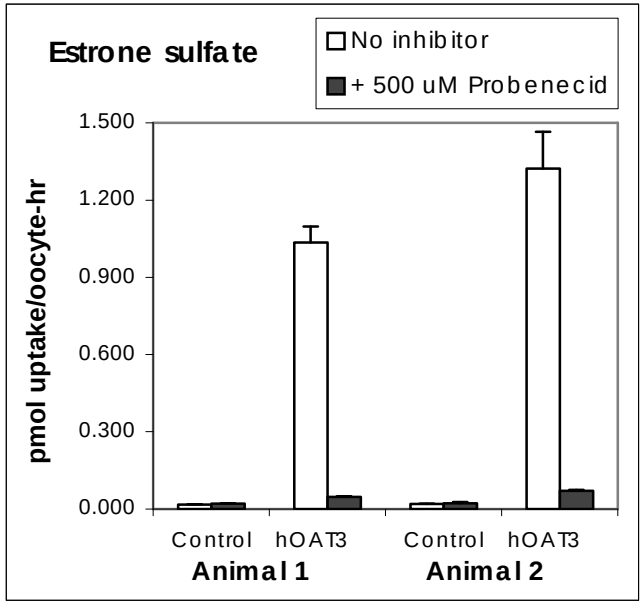


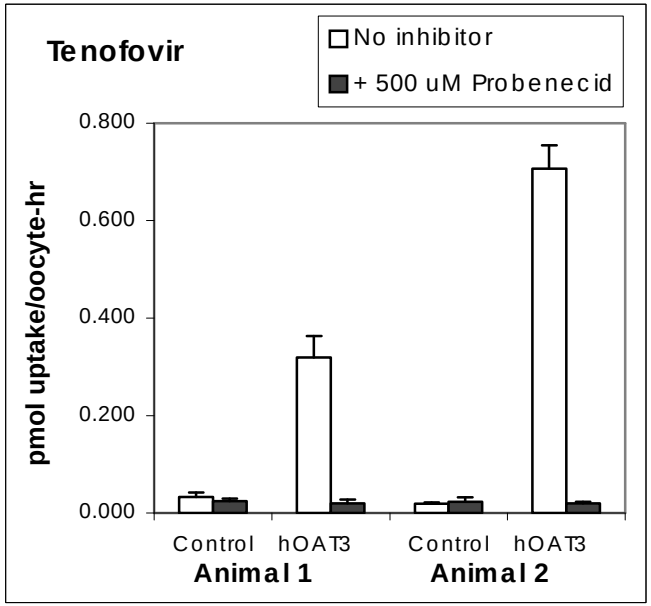




Report Title:	Study Type	Test Article	Report Number
In Vitro Interactions of Acyclic Nucleoside Phosphonate Analogs with Human Organic Cation and Anion Transporters	Drug-Drug Interaction	TFV	PC-103-2001

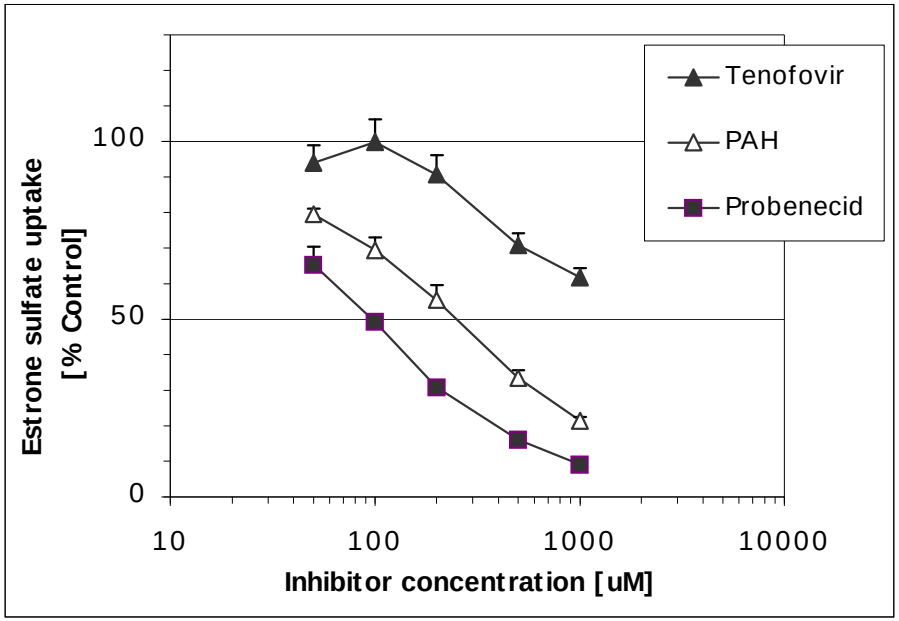
Results (OAT3): The figures below display the results of uptake studies of [^{3}H]TFV (10 µM) for human OAT3 in comparison to water-injected oocytes (Control) in the presence and absence of the control inhibitor, probenecid. The positive control substrate [^{3}H]estrone sulphate showed the expected high level of transport. TFV was shown to be a substrate for OAT3 transport.





Report Title:	Study Type	Test Article	Report Number
In Vitro Interactions of Acyclic Nucleoside Phosphonate Analogs with Human Organic Cation and Anion Transporters	Drug-Drug Interaction	TFV	PC-103-2001

The affinity of TFV for OAT3 was assessed indirectly through inhibition of the transport of [^{3}H]estrone sulphate in comparison to the effect of probenicid and para-aminohippurate (PAH). The results are shown in the figure below. No inhibition of OAT3 was observed in the presence of up to 100 μM TFV indicating a low affinity interaction between TFV and OAT3.



Conclusion: This study demonstrated that TFV was not a substrate for human cation transporters OCT1 and OCT2. TFV was shown to be a low affinity substrate for OAT3.

14.3.14. AD-120-2035: Effect of Cyclosporin A Pretreatment on Pharmacokinetics of TAF in Dogs

Report Title:	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on Pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Species:	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Intravenous infusion
Dose (mg/kg):	0.5
Sample	Plasma/PBMC
Assay:	LC-MS/MS

	Plasma		PBMC		
			- Phosphatase	+ Phosphatase	–
Parameter	**TAF**	**TFV**	**TFV**	**TFV**	**TFV-DP**
T_{max} (h)	0.41	0.90	0.48	2.32	6.00
C_{max} (µg/mL)	0.47	0.03	2.96	5.25	7.24
$t_{½}$ (h)	0.12	> 24	NC	> 24	> 24
AUC_{0-t} (µg•h/mL)	0.23	0.15	12.3	78.7	137
CL (L/h/kg)	2.23	NA	NA	NA	NA

AUC_{0-t} = area under the time-concentration curve from time zero to last measured time-point; C_{max} = maximum plasma concentration; CL = plasma clearance; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cell; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

Report Title:	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	2
Feeding Condition:	Fasted
Vehicle/Formulation:	0.9% sodium chloride in 50 mM ammonium acetate, pH 5.5
Assay:	LC-MS/MS

	Plasma		PBMC		
			- Phosphatase	+ Phosphatase	–
	TAF	TFV	TFV	TFV	TFV-DP
T_{max} (h)	0.14	0.67	1.00	1.00	8.70
C_{max} (µg/mL)	0.05	0.10	0.12	0.75	1.81
$t_{½}$ (h)	0.21	> 24	NC	> 24	> 24
AUC_{0-t} (µg•h/mL)	0.01	0.33	NA	10.8	31.2
F%	1.67	NA	NA	NA	NA

AUC_{0-t} = area under the time-concentration curve from time zero to last measured time-point; C_{max} = maximum plasma concentration; F = female; F% = bioavailability; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cell; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

Report Title:	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Species: Beagle dogs

Sex (M/F) / No. of Animals: M/3

Method of Administration: Oral gavage

Dose (mg/kg): 2

Co-administered CsA Dose (mg/kg): 75

Feeding Condition: Fasted

Vehicle/Formulation: 50 mM citrate, pH 5

Assay: LC-MS/MS

	Plasma (Pretreated with 75 mg Cyclosporin A)		PBMC (Pretreated with 75 mg Cyclosporin A)		
			- Phosphatase	+ Phosphatase	–
	TAF	TFV	TFV	TFV	TFV-DP
T_{max} (h)	0.25	1.00	1.00	1.00	3.00
C_{max} (µg/mL)	0.39	0.05	2.10	4.91	3.15
$t_{½}$ (h)	0.15	> 24	> 24	> 24	> 24
AUC_{0-t} (µg•h/mL)	0.15	0.38	9.39	75.8	59.5
F%	16.6	NA	NA	NA	NA

AUC_{0-t} = area under the time-concentration curve from time zero to last measured time-point; C_{max} = maximum plasma concentration; F = female; F% = bioavailability; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cell; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

14.3.15. AD-236-2003: Assessment of Inhibition of Human P-gp and BCRP by EVG, FTC, and TFV In Vitro

Report Title:	Study Type	Test Article	Report Number
In Vitro Inhibition of Human P-gp and BCRP by Elvitegravir, Emtricitabine and Tenofovir	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TFV	AD-236-2003

Methods: The inhibition of the ATP-Binding Cassette (ABC) efflux P-glycoprotein (P-gp) transporter and breast cancer resistance protein (BCRP) by EVG, COBI, FTC, and TFV was assessed in vitro using the MDCKII cells expressing individual transporters and fluorescent model substrates. Verapamil was the positive control inhibited P-gp activity and fumitremorgin C was the positive control inhibitor in BCRP inhibition assay.

Test Compound	Efflux Transporter IC_{50} (µM)	
	P-gp	BCRP
Elvitegravir	69.7 ± 5.4	88.9 ± 16.0
Emtricitabine	> 100	> 100
Tenofovir	> 1000	> 100
Cobicistat	36 ± 10	59 ± 28
Verapamil	5.2 ± 1.2	NA
Fumitremorgin C	NA	0.37 ± 0.18

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; MDCKII = Madin-Darby canine kidney cells; TFV = tenofovir; NA = not applicable

14.3.16. AD-236-2004: Bidirectional Permeability of EVG, FTC, TFV, and COBI through Monolayer of P-gp (MDR1)-Overexpressing Cells

Report Title:	Study Type	Test Article	Report Number
Bidirectional Permeability of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) through Monolayers of P-glycoprotein Over-expressing Cells	Drug-drug interaction (in vitro)	TFV	AD-236-2004

Bidirectional Permeability of TFV Through MDR1 Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
MDCKII-WT	Cell-Free	9.8	88	13.3	–	13.3	–
	Forward	9.5	104	0.18	0.11	0.15	1.5
	Reverse	13.1	95	0.19	0.25	0.22	
MDCKII-MDR1	Forward	16.0	94	0.10	0.12	0.11	1.0
	Reverse	16.8	92	0.07	0.15	0.11	
MDCKII-MDR1 (10 µM CsA)	Forward	13.2	83	0.12	0.07	0.10	1.5
	Reverse	11.0	82	0.14	0.15	0.14	

CsA = cyclosporin A; MDCKII = Madin-Darby canine kidney cells; MDR1 = P-glycoprotein (P-gp, ABCB1 gene product); P_{app} = apparent permeability; TFV = tenofovir; WT = wild type

14.3.17. AD-236-2005: Bidirectional Permeability of EVG, FTC, TFV, and COBI through Monolayers of BCRP-Overexpressing Cells

Report Title:	Study Type	Test Article	Report Number
Bidirectional Permeability of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) through Monolayers of BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TFV	AD-236-2005

Bidirectional Permeability of TFV Through BCRP Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
MDCKII-WT	Cell-Free	9.8	88	13.3	–	13.3	–
	Forward	9.5	104	0.18	0.11	0.15	1.5
	Reverse	13.1	95	0.19	0.25	0.22	
MDCKII-BCRP	Forward	12.1	92	0.17	0.14	0.16	2.3
	Reverse	13.1	81	0.40	0.32	0.36	
MDCKII-BCRP (10 µM CsA)	Forward	11.2	91	0.18	0.21	0.19	3.4
	Reverse	11.2	97	0.85	0.45	0.65	

BCRP = breast cancer resistance protein; CsA = cyclosporin A; MDCKII = Madin-Darby canine kidney cells; P_{app} = apparent permeability; TFV = tenofovir; WT = wild type

14.3.18. AD-236-2006: Assessment of Inhibition of Human OATP1B1 and OATP1B3 by FTC and TFV

Report Title:	Study Type	Test Article	Report Number
In Vitro Inhibition of Human OATP1B1 and OATP1B3 by Emtricitabine and Tenofovir	Drug-drug interaction (in vitro)	FTC, TFV	AD-236-2006

Methods The inhibition potential of test compounds of human OATP1B1 and OATP1B3 was assessed in Chinese hamster ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3. The test compounds and positive control compound were diluted in assay buffer containing 2 µM Fluo 3. Following removal of media containing Fluo 3, the cells were immediately analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm. Rifampicin was used as positive control.

Test Compound	Influx Transporters IC_{50} (µM)	
	OATP1B1	OATP1B3
Elvitegravir	> 2	0.44 ± 0.22
Emtricitabine	> 100	> 100
Tenofovir	> 100	> 100
Cobicistat	3.50 ± 0.72	1.88 ± 0.76
Rifampicin	1.3 ± 0.8	3.0 ± 0.7

FTC = emtricitabine; OATP = organic anion transporting polypeptide; TFV = tenofovir

14.3.19. AD-236-2007: Assessment of Inhibition of EVG, FTC, TFV, and COBI with Human OAT1, OAT3 and MRP4 Transporters

Report Title:	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Quad Components with Human OAT1, OAT3 and MRP4 Transporters	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TDF	AD-236-2007

Methods: The potential for test compounds to inhibit the human organic anion uptake transporters (OAT1 and OAT3) and the apically expressed human multidrug resistance related protein 4 (MRP4) was assessed in vitro. OAT1 and OAT3 cellular uptake assay was performed on Chinese hamster ovary (CHO) cells and human embryonic kidney (HEK293) cells with FlpIn technology (FlpIn293) stably transfected with OAT1 and OAT3, respectively. In MRP4 vesicular transport assay, the test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) in the absence or presence of ATP. The amount of substrate inside the cells was determined by liquid scintillation counting.

		Emtricitabine		Elvitegravir		Cobicistat		Tenofovir	
Transporter	Probe Substrate	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)
OAT1	PAH	> 100	40	> 20	No Inhibition	> 100	140 activation	33.8	NA
OAT3	E3S	> 100	No Inhibition	> 20	14	> 100	No Inhibition	770	NA
MRP4	E217βG	> 100	No Inhibition	> 20	19	20.7	~92	> 1,000	NA

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; TFV = tenofovir; PAH = ^{3}H- para-aminohippuric acid; E3S = ^{3}H-estrone-3-sulfate;
E217βG = estradiol-17-beta-glucuronide; OAT = organic anion transporter; NA = not applicable
Note: Maximum concentrations used for elvitegravir, emtricitabine, cobicistat and tenofovir were 20, 100, 100, and 1000 µM, respectively.

14.3.20. AD-236-2008: Assessment of Inhibition of EVG, COBI, FTC, and TFV with Human OCT1 and BSEP Transporters

Report Title:	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Stribild Components with Human OCT1 and BSEP Transporters	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TFV	AD-236-2008

Methods The potential for test compounds to inhibit the human organic cation uptake transporter (OCT1) and bile salt export pump (BSEP) was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells. In OCT2 cellular uptake assay, plated cells were washed with Krebs-Henseleit buffer and were then exposed for 10 minutes to the same buffer containing [^{14}C]-tetraethylammonium chloride (TEA) substrate (3.6 µM) and test compounds. In BSEP vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate, taurocholate (2 µM) in the absence or presence of ATP. The amount of substrate inside the cells was determined by liquid scintillation counting for both assays.

	Elvitegravir		Emtricitabine		Tenofovir		Cobicistat		Ritonavir	
Transporter	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)	IC_{50} (µM)	Maximum inhibition (%)
OCT1	> 20	30	>100	No Inhibition	> 100	No Inhibition	14.7	76	~20	49
BSEP	> 20	33	>100	No Inhibition	> 100	No Inhibition	6.5	97	1.8	95.3

BSEP = bile salt export pump; COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; OCT = organic cation transporter; Stribild = fixed-dose combination of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate; TFV = tenofovir
Note: Maximum concentrations used for elvitegravir, emtricitabine, cobicistat, tenofovir and ritonavir were 20, 100, 100, 100, and 20 µM, respectively.

14.3.21. AD-236-2011: Interaction of FTC and TFV with Human OCT2 Uptake Transporters

Report Title:	Study Type	Test Article	Report Number
In vitro Interaction Study of Emtricitabine and Tenofovir with the Human OCT2 Uptake Transporter	Drug-drug interaction (in vitro)	FTC, TFV	AD-236-2011

Methods: The potential for test compounds that as substrates for the renal uptake transporter organic cation transporter (OCT2) was assessed in vitro using wild-type and transfected Chinese Hamster Ovary (CHO) cells. The transporter specific uptake of emtricitabine and TFV was determined using cells overexpressing the OCT2 uptake transporters as well as the control (parental) cells. The test articles were incubated at 37±1°C at final concentrations of 1 and 10 µM. The amount of substrate inside the cells was determined by LC-MS/MS method.

	Fold Accumulation	
Condition (Concentration [µM]/Time [min])	Emtricitabine	Tenofovir
1/2	2.35[a]	1.24
1/20	1.12	1.37
10/2	1.10	0.82
10/20	1.82	0.98

FTC = emtricitabine; LC/MS = high performance liquid chromatography coupled to tandem mass spectrometry; OCT = organic cation transporter; TFV = tenofovir
a Considered outlier

14.4. EVG/COBI/FTC/TFV

14.4.1. AD-236-2001: Inhibition Potential of EVG, FTC, TFV, and COBI with Human OCT2 and MATE1 Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition Studies of Elvitegravir, Emtricitabine, Tenofovir, and Cobicistat (Quad) with Human OCT2 and MATE1 Transporters	Drug-drug interaction (in vitro)	EVG, COBI, FTC, TFV	AD-236-2001

Methods The potential for EVG, COBI, FTC, and TFV to inhibit the human organic cation uptake transporters OCT2 and multidrug and toxin extrusion transporter MATE1 was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells lines stably expressing human OCT2 or MATE1 protein. The amount of cell-associated radioactivity was determined by liquid scintillation counting. IC_{50} values were calculated by analysis of the concentration-dependent reduction of the fractional transport activity by the test compounds, with 100% transport activity being that seen with the vehicle control. IC_{50} values were determined by nonlinear regression using GraphPad Prism 5.0.

Inhibitor or Enhancer	Transporter	IC_{50} (µM)	Maximum inhibition at 100 µM (% of control)
Elvitegravir	OCT2	>20	31
	MATE1	2.0	98
Emtricitabine	OCT2	>100	18
	MATE1	>100	ND
Tenofovir	OCT2	>300	ND
	MATE1	>300	20
Cobicistat	OCT2	14.4	70
	MATE1	1.87	98

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; ND = not determined; TAF = tenofovir alafenamide; TFV = tenofovir
Note: Maximum concentration tested was 300 µM for tenofovir, 100 µM for emtricitabine and cobicistat, and 20 µM for elvitegravir

14.4.2. AD-236-2010: Interaction of FTC with Human OAT1 and OAT3 Transporters

Report Title	Study Type	Test Article	Report Number
In vitro Interaction Study of Emtricitabine with the Human OAT1 and OAT3 Uptake Transporters	Drug-drug interaction (in vitro)	FTC	AD-236-2010

Methods The potential for test compounds that as substrates for the renal uptake transporters organic anion transporter (OAT1 and OAT3) was assessed in vitro using wild-type and transfected cell-lines. The transporter specific uptake of emtricitabine was determined using cells overexpressing the OAT1 and OAT3 uptake transporters as well as the control (parental) cells. The test article was incubated at 37±1°C at final concentrations of 1 and 10 µM. An additional experiment was performed in OAT3 overexpressing cells in the presence and absence of probenecid, a known inhibitor of the transporter. The amount of substrate inside the cells was determined by LC/MS method.

Transporter	Condition (µM/min)	Fold Accumulation
OAT1	1/2	1.59
	1/20	0.66
	10/2	1.51
	10/20	1.45
OAT3	1/2	2.09
	1/20	1.82
	10/2	2.32
	10/20	2.13
	10/2	2.71 (Emtricitabine Alone)
	10/2	1.02 (Emtricitabine + Probenecid)

FTC = emtricitabine; LC/MS = high performance liquid chromatography coupled to tandem mass spectrometry; OAT = organic anion transporter

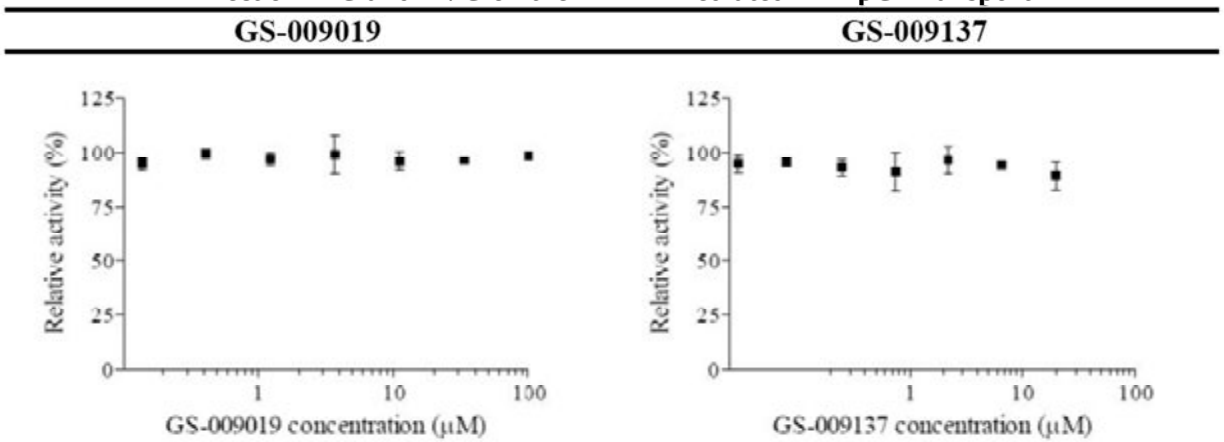
14.4.3. AD-236-2012: Assessment of Interaction of EVG and FTC with Human MRP2 Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Inhibition of Human MRP2 ABC (Efflux) Transporter by Stribild Components Elvitegravir and Emtricitabine	Drug-drug interaction (in vitro)	EVG, FTC	AD-236-2012

Methods The inhibition potential of test compounds with human MRP2 ABC (efflux) transporters was assessed in vitro using MRP2 containing vesicles and model substrate estradiol-17-beta-glucuronide (E217bG). The vesicular transport assay was performed with cell membrane vesicles containing the human MRP2 efflux transporter. The MRP2 transporter was expressed in Spodoptera frugiperda (Sf9) ovarian cells. The isolated membrane vesicles were prepared and characterized. The specificity of the interaction was confirmed using control membrane vesicles in the negative control experiment. The concentration of test articles was 0 and the highest applied test article concentration. The amount of substrate inside the filtered vesicles was determined by liquid scintillation.

Results Elvitegravir and emtricitabine did not influence the MRP2-mediated E217βG transport when tested at concentrations up to 20 µM and 100 µM, respectively.

Effect of FTC and EVG on the MRP2-Mediated E217βG Transport



EVG = elvitegravir (GS-009137), FTC = emtricitabine (GS-009019); MRP2 = multidrug resistance-associated protein-2 (ABCC2, cMOAT); Stribild = fixed-dose combination of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate

14.4.4. AD-236-2013: Interaction of FTC with Human MRP2 Transporters

Report Title	Study Type	Test Article	Report Number
In Vitro Interaction Study of Emtricitabine with the Human MRP2 ABC (Efflux) Transporter	Drug-drug interaction (in vitro)	FTC	AD-236-2013

Methods The potential for test compound that as substrates for human MRP2 ABC transporter was assessed in vitro using MRP2 containing membrane vesicles. Transporter specific accumulation of emtricitabine in MRP2 vesicles was investigated at 2 concentrations (1 and 10 µM) and at 2 incubation times (2 and 20 minutes). Emtricitabine was incubated at 37±1°C at final concentrations of 1 and 10 µM. The amount of emtricitabine accumulated in the vesicles was determined by LC/MS method. MRP2-mediated E217βG transport in the presence or absence of 100 µM benzbromarone was carried out as a positive control for MRP2 function.

Transporter	Condition (µM/min)	Fold Accumulation
MRP2	1/2	ND
	1/20	ND
	10/2	1.06
	10/20	0.99

E217βG = estradiol-17-beta-glucuronide; FTC = emtricitabine; MRP2 = multidrug resistance-associated protein-2 (ABCC2, cMOAT); ND = not determined
Most of the samples from the 1 µM group were below the limit of quantification therefore fold accumulations could not be calculated

15. PHARMACOKINETICS: OTHER

There are no additional studies to report under this heading.